ALTERATIONS IN BRAIN STRUCTURE AND FUNCTION IN CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDER

by

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Abstract

Fetal Alcohol Spectrum Disorder (FASD) can occur when a mother drinks alcohol during pregnancy. The full spectrum of adverse effects on the brain induced by prenatal alcohol exposure ranges from mild to severe brain dysfunction in executive functions, learning, memory, social communication, and sensory-motor skills. The goals of this thesis were to assess the functional outcomes of children with FASD using psychometric testing and eye movement control tasks and relate these to measures obtained from diffusion tensor imaging (DTI). Results from the eye movement studies successfully differentiated those with FASD from controls on both sensory-motor and behavioural outcomes. Children with FASD showed deficits in response inhibition, working memory, saccadic reaction time, and saccade metrics on three eye movement tasks. A sexually dimorphic impact of prenatal alcohol exposure on eye movement control was also found. The psychometric tests revealed deficits in set shifting, response inhibition, selective and sustained attention, working memory, and visuospatial processing. The DTI results revealed significantly higher mean diffusivity in the splenium of the corpus callosum. These measures were then correlated to one another to search for common brain pathways utilized to complete these tasks. Working memory measures obtained from the memory-guided eye movement task significantly correlated with three psychometric tests which measured working memory in the FASD group. The prosaccade eye movement task which measures basic sensory-motor processing was also correlated with a visuospatial processing psychometric task in the FASD group. Finally, response inhibition measures from the eye movement tasks correlated with response inhibition measures obtained from the psychometric testing in the FASD group. Additionally, eye movement inhibition measures correlated negatively to fractional anisotropy and positively to mean diffusivity of the splenium in the control, but not the FASD group.
Therefore, brain function of typically developing controls and children with FASD can be successfully assessed using eye movement tasks, psychometric testing and DTI. These functional measures help identify specific brain regions affected by prenatal alcohol exposure which can lead to more specific interventions. These findings can also help streamline the diagnostic process by pointing to efficient and effective tools that differentiates children with FASD from controls.
Co-Authorship

The research described in this thesis was conducted by Angelina Paolozza under the supervision of Dr. James Reynolds. Angelina Paolozza wrote all first drafts of all chapters and was lead author on all subsequent drafts. Chapters 2 and 3 were in collaboration with Dr. Douglas Munoz.

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“Learn from yesterday, live for today, hope for tomorrow. The important thing is to not stop questioning.”

— Albert Einstein, Relativity: The Special and the General Theory
Statement of Originality

I hereby certify that all of the work described within this thesis is the original work of the author. Any published (or unpublished) ideas and/or techniques from the work of others are fully acknowledged in accordance with the standard referencing practices.

Angelina E. Paolozza

(January, 2015)
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<th>Full Form</th>
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<tbody>
<tr>
<td>ARBD</td>
<td>Alcohol related birth defects</td>
</tr>
<tr>
<td>ARND</td>
<td>Alcohol related neurodevelopmental disorder</td>
</tr>
<tr>
<td>ADHD</td>
<td>Attention deficit hyperactivity disorder</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>COV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>dlPFC</td>
<td>Dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>CN</td>
<td>Caudate nucleus</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<tr>
<td>$\lambda$</td>
<td>Eigenvalue</td>
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<tr>
<td>IOM</td>
<td>Institute of Medicine</td>
</tr>
<tr>
<td>IML</td>
<td>Internal medullary lamina</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
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<tr>
<td>FASD</td>
<td>Fetal alcohol spectrum disorder</td>
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<td>FAS</td>
<td>Fetal alcohol syndrome</td>
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<tr>
<td>FAEE</td>
<td>Fatty acid ethyl esters</td>
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<td>FA</td>
<td>Fractional anisotropy</td>
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<tr>
<td>FEF</td>
<td>Frontal eye fields</td>
</tr>
<tr>
<td>FP</td>
<td>Fixation point</td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid-attenuated inversion recovery</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>LIP</td>
<td>Lateral intraparietal cortex</td>
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<tr>
<td>MEG</td>
<td>Magnetoencephalography</td>
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<tr>
<td>MD</td>
<td>Mean Diffusivity</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>NEPSY</td>
<td>Neuropsychological Assessment</td>
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<td>pFAS</td>
<td>Partial fetal alcohol syndrome</td>
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<tr>
<td>PEth</td>
<td>Phosphatidylethanol</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PAE</td>
<td>Prenatal alcohol exposure</td>
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<tr>
<td>PCA</td>
<td>Principal components analysis</td>
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<tr>
<td>ROI</td>
<td>Region of interest</td>
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<tr>
<td>SEF</td>
<td>Secondary eye fields</td>
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<tr>
<td>SRT</td>
<td>Saccadic reaction time</td>
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<tr>
<td>SES</td>
<td>Socioeconomic status</td>
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<tr>
<td>SNp</td>
<td>Substantia nigra pars compacta</td>
</tr>
<tr>
<td>SC</td>
<td>Superior colliculus</td>
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<tr>
<td>TBSS</td>
<td>Tract-based spatial statistics</td>
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<td>WJ</td>
<td>Woodcock Johnson</td>
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<tr>
<td>WRMT</td>
<td>Woodcock Reading Mastery Test- Revised</td>
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<tr>
<td>WMTB-C</td>
<td>Working Memory Test Battery for Children</td>
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Chapter 1

General Introduction

“Everybody is a genius. But if you judge a fish by its ability to climb a tree, it will live its whole life believing that it is stupid.” – Albert Einstein

1.1 Research Problem

The full spectrum of adverse effects induced by prenatal alcohol exposure is collectively referred to as Fetal Alcohol Spectrum Disorder (FASD), and includes several diagnostic subgroups. These subgroups include fetal alcohol syndrome (FAS) and partial fetal alcohol syndrome (pFAS), which present with facial features, growth deficiency, and central nervous system dysfunction; and alcohol related neurodevelopmental disorder (ARND) which presents with central nervous system dysfunction (Chudley et al. 2005). The developing brain is the principal target organ for prenatal alcohol exposure, which may result in intellectual, neurological and/or behavioural abnormalities. These abnormalities produce a wide variety of mild to severe dysfunctions in both primary (executive functions, visuospatial processing, motor skill, etc.) and secondary (neuropsychiatric and maladaptive outcomes) processes (Kodituwakku 2009; Mattson et al. 2011; Rasmussen 2005; Rasmussen et al. 2008). Despite widespread recognition of the adverse consequences associated with prenatal alcohol exposure, FASD remains the most common preventable developmental disorder in the world. Recent research indicates that FASD may occur as frequently as 2-5 per 100 live births (May et al. 2009). Current cost estimates for FASD in Canada ranges from $5.3-$7.6 billion annually which creates an enormous burden on the child, family, and society (Stade et al. 2009; Thanh and Jonsson 2009).
One of the most significant problems associated with FASD diagnosis is that in the absence of facial features, identification of children prenatally exposed to alcohol becomes increasingly difficult, especially if alcohol exposure cannot be confirmed; therefore, accurate assessment tools need to be developed to quickly and correctly identify these children and close this diagnostic gap. Several different types of biomarkers for prenatal alcohol exposure are being investigated that can be used at birth to identify infants prenatally exposed to alcohol. These include testing for fatty acid ethyl esters (FAEEs) found in meconium and hair, phosphatidylethanol (PEth) found in blood samples, and facial imaging to identify the facial features associated with FAS and pFAS. However, these are only an indication of exposure and provide no real information on the functional outcomes of the individual child. Therefore, new techniques are needed that can assess the functional outcome in children prenatally exposed to alcohol. These ‘functional biomarkers’ could be used to screen children at risk to determine whether or not a full diagnostic assessment is recommended. Some techniques which hold promise include magnetic resonance imaging (MRI), eye movement control tasks, and psychometric testing. This thesis was conducted to evaluate these three techniques in a large group of children with FASD to search for accurate and efficient functional biomarkers of prenatal alcohol exposure. It was hypothesized that these measures would successfully differentiate children with FASD from control children.
1.2 Diagnosis of FASD

1.2.1 Early Diagnosis

Early diagnosis is of the utmost importance in FASD as it can lead to early interventions for both the mother and child. Early identification has been found to decrease secondary disabilities like mental health problems, unemployment, institutionalization, and inappropriate behaviour in those with FASD (Rasmussen, Andrew, Zwaigenbaum, & Tough 2008; Streissguth et al. 2004). Unfortunately, many cases of FASD go undiagnosed or are misdiagnosed (Abel 1995). This creates a missed opportunity to legitimately identify and help an exposed infant or child (Marcellus 2007). For infants born with facial features early assessment is important as some discriminating facial features of FAS and pFAS can become less recognizable with increasing age (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). However, for children under the age of 6 who do not have any of the FASD facial features, a diagnosis is less likely. This may be because these children are too young to participate in higher level cognitive testing (Astley 2010). Together, these inaccuracies could hinder efforts to allocate sufficient social and health care services to these vulnerable children and preclude accurate assessment of primary prevention efforts and effective screening tools, which are desirable for early diagnosis, especially for those children who do not present with facial features.

1.2.2 Canadian Guidelines

Currently in Canada there are three approaches to diagnosis of FASD, with varying degrees of stringency and thoroughness. Multidisciplinary diagnosis is recommended and requires input from a case coordinator, physician, psychologist, occupational therapist, and speech-language pathologist (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). Chudley et al. (2005) recommend a diagnostic process that consists of screening and referral,
physical examination and differential diagnosis, neurobehavioural assessment, and treatment with follow-up. Screening for prenatal alcohol exposure should be completed in all pregnant and post-partum women. Referral for follow-up should be made if the infant or child presents with any facial features or there is evidence of prenatal alcohol exposure. Following a referral the child should be given a physical examination and all other diseases and disorders should be excluded. Many disorders such as Williams syndrome, maternal phenylketonuria, Dubowitz syndrome, and fetal anticonvulsant syndrome can all mimic several of the same characteristics that present in FASD (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). The Institute of Medicine (IOM) guidelines and the 4-digit diagnostic code (described below) are the two main approaches used by clinics in Canada to diagnose FASD. These two approaches have different criteria but overlap on many of the basic principles.

The IOM recommendations for diagnosis were first published in 1996 (Stratton et al. 1996). To receive a diagnosis of FAS the IOM method requires the presence of a characteristic pattern of facial abnormalities, evidence of growth restriction and central nervous system abnormalities, with or without confirmed alcohol exposure. pFAS requires confirmed alcohol exposure, one or two facial features, and either growth restriction, central nervous system damage, or evidence of a complex pattern of behaviour or cognitive abnormalities that are inconsistent with developmental level. To qualify for a conclusion of alcohol related effects there must be a history of prenatal alcohol exposure plus clinical evidence that has linked maternal alcohol ingestion to an observed effect (i.e. learning disabilities, poor impulse control, social problems, etc.). There are two subgroups included in this category: alcohol related birth defects (ARBD) which requires the presence of congenital anomalies; and ARND which requires the
presence of central nervous system dysfunction and/or a pattern of behaviour or cognitive abnormalities that are inconsistent with developmental level.

The 4-digit code has many of the same diagnostic subtypes but instead uses a four digit code to dictate which specific subtype is used (Astley and Clarren 1999). The four digit code assesses growth deficiency, facial features, central nervous system damage, and alcohol exposure, each on a four point scale. A score of one indicates no feature and a score of four indicates severe presentation. To receive a diagnosis of FAS the patient must exhibit mild to severe growth restriction, all three prominent facial features, moderate to severe central nervous system damage, and either unknown or confirmed alcohol exposure. To receive a diagnosis of pFAS the patient may exhibit variable (absent to severe) growth restriction, moderate to severe facial features, moderate to severe central nervous system damage, and confirmed alcohol exposure. There are many other subtypes under the four digit code that resemble ARND including static encephalopathy and neurobehavioural disorder. To receive a diagnosis of an ARND equivalent the patient may present with variable (absent to severe) growth restriction, variable (absent to mild) facial features, mild to severe central nervous system damage, and confirmed alcohol exposure. The 4 digit code does not recognize ARBD as a FASD diagnosis. In 2005, Chudley et al. published the Canadian guidelines for FASD diagnosis that resulted from a consensus conference funded by the Public Health Agency of Canada (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). This harmonized the IOM and 4 digit code approaches together.

As previously mentioned both diagnostic approaches investigate growth deficiency in children suspected to have FASD. All approaches consider prenatal and/or postnatal height or weight less than or equal to the 10th percentile to be a deficiency. However, the child’s parental
height and weight, genetic potential and other conditions must also be taken into consideration, in order to identify and separate the growth deficiency characteristic of alcohol’s teratogenicity from the child’s genetics and postnatal environment.

The facial phenotype is one of the most common and distinctive features of heavy prenatal alcohol exposure. The face of FAS is distinguished by the simultaneous presentation of a smooth philtrum, thin upper lip and small palpebral fissure lengths. These features are highly specific (99%) and sensitive (100%) (Astley and Clarren 2001). The facial features are measured manually using direct measurements or using computerized analysis of a digital photograph. The computerized method is more accurate and can be completed using a standard digital camera and is therefore accessible for all clinicians (Astley & Clarren 2001). While these facial features are highly useful for identifying those with FASD, not every child with brain damage due to prenatal alcohol exposure presents with these features.

A neurological and behavioural assessment accompanies FASD diagnosis to search for central nervous system damage due to the teratogenic effects of alcohol. The information obtained in these assessments varies widely from structural scans to cognition and communication skills. It is recommended that the assessment includes basic and complex tasks in many domains of brain function as separate entities to build a proper profile of functioning. Each diagnostic approach has a difference cut off to determine if a domain is impaired. The Canadian guidelines dictate that the participant must be 2 or more standard deviations below the mean on 3 or more domains to receive a diagnosis. The 4 digit code and the IOM require structural/neurological and/or severe brain dysfunction to receive a diagnosis.

Presently, detection of prenatal alcohol exposure is largely based on maternal self-reporting or documented history (Burd and Hofer 2008). All diagnostic approaches require
confirmed prenatal alcohol exposure for a diagnosis of pFAS or ARND. A child is often identified as a possible case of FASD during early childhood (around age 5) when cognitive and behavioural issues become more apparent. In North America, many of these children are in foster care due to circumstances such as parental substance abuse. At this point, determining maternal alcohol use is either difficult to obtain due to the mother’s absence and/or lacks accuracy (Del Boca and Darkes 2003). Even if the mother can be found or given a questionnaire soon after giving birth, Del Boca and Darkes (2003) report that often the mothers will lie on these questionnaires. This is typically due to social contexts, respondent characteristics, and task attributes. Response behaviour is influenced by a variety of social context factors such as cultural prescriptions, stigma, subcultural norms, and interpersonal situations. Providing anonymity and confidentiality help mitigate these social fears but do not completely eliminate them as drinking during pregnancy is highly stigmatized. Respondent characteristics such as demographic identifications, reference groups, personality, and transitory states can also affect alcohol self-report. For example, dependence severity, recovery stage, sobriety, and withdrawal state have all been shown to affect response validity (Babor et al. 2000). However, very little research has been conducted to help quantify and diminish these effects. Task attributes depend on mode of administration, complexity of task, clarity, and instrument design. Many instruments must be validated in order to ensure they are understandable and accurate, especially with questions based on drinking quantity and understanding the size of a ‘standard’ drink. The instruments currently used by clinicians and researchers each have their own strengths and weaknesses and are only appropriate in certain situations. This is why more widespread measures need to be developed in order to accurately assess prenatal alcohol exposure.
1.2.3 Diagnostic Challenge

FASD presents with a significant diagnostic challenge due to the wide spectrum of deficits that may occur and the dependence on obtaining accurate information on the amount and timing of alcohol exposure. Additionally, most of the deficits are not specific to FASD itself (Mattson et al. 2012; May et al. 2013; Ware et al. 2014). The diagnosis process itself is quite lengthy and professionals from multiple disciplines are required. This process also requires specialized training and is quite expensive. Finally, diagnosis and criteria can vary widely from clinic to clinic within Canada and the world. Therefore, an objective screening tool that could help streamline the process for referral would be both useful and cost effective. Measurement of eye movement control is a powerful tool for assessing cognitive, sensory and motor function simultaneously and therefore provides such a potential screening tool (Leigh and Kennard 2004; Leigh and Zee 2006; Ramat et al. 2007). Eye movements can be tested using customized tasks to allow for specific central nervous system functions to be assessed. These tasks can also be administered with minimal training while also being objective, reproducible, and accurate. In order for this tool to be used effectively as a screening tool it must first successfully differentiate those with FASD from controls and other neurobehavioural disorders. It must also test many of the same constructs assessed using the current diagnostic criteria.
1.3 Psychometric Assessment Tools

1.3.1 Psychometric Assessment Background

Alcohol is a teratogen that can alter the developing brain in a variety of ways ranging from gross structural anomalies to subtle alterations in the concentrations of neurochemicals which can lead to altered brain function (West 1986). The ability to detect structural, neurological, and functional CNS abnormalities is dependent on the sensitivity of current measurement tools, which continue to improve over time. Some challenges to collecting psychometric data are that not all structural or neurological abnormalities result in measurable dysfunction and not all functional abnormalities are due to underlying brain damage. Additionally, some functional abnormalities result from adverse postnatal environmental factors and are transient in nature; therefore, separating these from damage due to prenatal alcohol exposure can be difficult.

1.3.2 Psychometric Measures

1.3.2.1 Neuropsychological Assessment (NEPSY) II

The NEPSY-II tool is a standardized psychometric test used to assess multiple domains of cognitive functioning in children aged 3-16 years (Korkman et al. 2007). It consists of 32 subtests that are divided into six cognitive domains: attention and executive functioning, language, memory and learning, sensorimotor, social perception, and visuospatial processing. Of the 32 subtests, 5 were chosen for the current thesis based on previous studies and to keep the length of the study protocol to a minimum. These 5 include: (i) animal sorting which was chosen to assess the participant’s ability to formulate basic concepts and successfully use those concepts to shift between categories; (ii) auditory attention and response set were used to test attention selectivity and vigilance (auditory attention) as well as attention shifting and vigilance (response
(iii) inhibition was selected to assess multiple levels of inhibitory skill including the ability to inhibit automatic responses in favor of novel responses and the ability to switch between response types; (iv) memory for names was chosen because it assesses ability to retain names in short term memory and also includes a delayed component which assesses long term memory for names; and (v) arrows were selected to assess visuospatial processing of line orientation. The variety of domains tested allows for examination of different types of impairments that may co-occur together. This battery of tests also provides a broad description of impairments that can occur due to prenatal alcohol exposure.

1.3.2.2 Working Memory Test Battery for Children (WMTB-C)

The WMTB-C is designed to assess working memory in children aged 5-15 years. It is based on the three components of Baddeley and Hitch’s model of working memory (Baddeley and Hitch 1974; Gathercole and Pickering 2001). This battery consists of 9 subtests that are divided into tests of central executive, phonological loop and visuospatial sketchpad. Of the 9 subtests 2 were chosen for the current thesis based on previous research and to keep the length of the study protocol to a minimum. These include: (i) digit recall which was used to assess verbal working memory and the phonological loop; and (ii) block recall was selected to assess spatial working memory and the visuospatial sketchpad. Using these two subtests allows for two of the three components of working memory to be examined in a short duration.

1.3.2.3 Woodcock Reading Mastery Test- Revised (WRMT)

The WRMT is designed to assess reading ability in participants aged 5-75+ years. It consists of 6 subtests designed to measure beginning reading, basic reading skills and reading comprehension skills. Of the 6 subtests only 1 was chosen to represent reading ability in the
FASD group. Word Identification was chosen because it gives a measure of basic reading skills across all age ranges.

1.3.2.4 Woodcock Johnson (WJ) III

The WJIII is designed to assess a broad range of cognitive skills in participants aged 5-75+ years. It consists of 22 subtests that measure reading, mathematics, language, academic knowledge, and writing. Of the 22 subtests only 1 was chosen to give a measure of mathematical ability. Quantitative Concepts measures both math knowledge such as symbols and vocabulary as well as quantitative reasoning.

1.3.3 Psychometric Assessment and FASD

Due to the wide variety of central nervous system deficits seen in those with FASD, early work was aimed at developing a tool that specifically tested for these deficits. The Fetal Alcohol Behaviour Scale was developed in 1996 to describe the behavioural profile of FASD. This was accomplished by interviewing 134 caregivers and deriving a scale based on their answers (Streissguth et al. 1998). This type of psychometric tool can be used by any health care provider as a tool to help identify children with FASD. As previously mentioned, due to the lack of facial features, those with ARND are increasingly difficult to diagnose. Therefore, the ARND Diagnostic Criteria Checklist was created to diagnose those patients that do not meet the diagnostic criteria for FAS. This was based on past research as well as caregiver’s descriptions (Greenbaum et al. 2002). Finally, a third checklist has been developed to help distinguish those with FASD from those with attention deficit hyperactivity disorder (ADHD). Since ADHD is highly comorbid with FASD the goal was to help distinguish the two groups to better characterize the neurobehavioural profile of those with FASD. This was accomplished by using caregiver report on the Child Behavioural Checklist (Nash et al. 2006). While these checklists
had reasonable success they were based on caregiver report which may not give a completely accurate picture of actual functioning of the child. Indeed, many studies have found mixed results when comparing caregiver rating scales to psychometric measures of cognitive functioning in those with FASD (Gross et al. 2014).

New approaches to improving FASD diagnosis have focused on using standardized psychometric testing to develop a profile. Indeed many studies have successfully differentiated those with FASD from controls using a variety of psychometric tests with large effect sizes (Kodituwakku 2009; Korkman et al. 2003; Olson et al. 1998; Pei et al. 2011b; Rasmussen et al. 2006; Rasmussen, Andrew, Zwaigenbaum, & Tough 2008; Rasmussen et al. 2012; Vaurio et al. 2011). These studies have found deficits in those with FASD on many different cognitive and behavioural measures including executive functioning, working memory, adaptive skills, communication, and visuospatial processing. Additionally, one study found fairly high (71.5-76.1%) classification accuracy between those with FASD compared to controls and those with ADHD using 11 psychometric tests (Vaurio et al. 2008). Another study used maternal factors such as demographics, physical, drinking, and childbearing to predict cognitive and behavioural characteristics of children with FASD (Jacobson et al. 1998). While psychometric testing has shown success at differentiating those with FASD from controls and other comorbid disorders, administration of the tasks is lengthy, can be interpreted incorrectly and the administrator can inadvertently introduce bias especially if they are not blinded to clinical group.
1.4 Eye Movement Control

1.4.1 Eye Movement Control Background

Saccades are rapid eye movements that redirect the line of sight to bring new images of interest onto the fovea of the retina. The fovea is located in the center of the macula region of the retina and is the area with the highest visual acuity. For the eyeball to make a saccade it must overcome forces imposed by orbital supporting tissue by contracting the extraocular muscles. To make a saccade this requires the phasic activation of neural activity in the eye movement control system to overcome viscous drag and elastic forces which requires tonic activation of the extraocular muscles to keep the eye in the new position (Leigh & Zee 2006). Each eye is surrounded by six extraocular muscles; four rectus muscles and two oblique muscles. Horizontal eye movements are controlled by the medial and lateral rectus muscles, which act together in adduction and abduction, respectively. While vertical eye movements require the coordinated action of the superior and inferior rectus muscles in combination with the superior and inferior oblique muscles, where elevation is due to the action of the superior rectus and inferior oblique, and depression is due to the inferior rectus and superior oblique (Purves et al. 2001).

Saccades include both involuntary and voluntary eye movements. Involuntary saccades are generally reflexive in nature and occur in response to the sudden appearance of a novel visual stimulus in one’s environment or to scan a visual scene. Voluntary saccades are purposeful eye movements to look at an object in one’s environment or toward a remembered target. Saccades can be described by the same basic main characteristics which include velocity, duration, waveform, trajectory, latency, and accuracy. Saccade velocity is the speed of the eye movement and amplitude is the distance of the saccade. Amplitude and velocity show a consistent relationship where the larger the eye movement the greater the peak velocity (Leigh & Zee
Saccade duration is also approximately linearly related to amplitude for smaller eye movements. This relationship between velocity, amplitude and duration is known as the main sequence. While saccade amplitude can be controlled, the velocity and duration are involuntary. The saccade velocity waveform can also be used to characterize saccades by examining the shape of the velocity and acceleration profile. The skewness of the velocity waveform can be examined to look for asymmetries between the acceleration and deceleration phases. The acceleration profile can also be used to measure peak acceleration and deceleration. Due to the large body of both functional and electrophysiological literature, these metrics can be examined to search for deficits in specific brain structures.

Other basic measures such as saccadic trajectory can also be measured. Saccade trajectory is not perfectly straight but instead saccades are generally recorded to initiate at some angle away from the target and, via an online correction mechanism (discussed below), the saccade is curved inward and therefore saccade trajectory is most frequently recorded as a curved line (Smit and Van Gisbergen 1990; Van der Stigchel et al. 2006). Saccade deviation can be used to measure trajectory by using a straight line from the beginning of the saccade to the endpoint and comparing this to a perfectly straight reference line. An abnormal trajectory can lead to inaccurate saccades. Additionally, inaccuracy can be caused by overshooting (hypermetric) or undershooting (hypometric) the target or object of interest. This inaccuracy is generally adjusted using small corrective saccades to reach the desired location (Leigh & Zee 2006).

Finally, saccadic reaction time (SRT) is another basic characteristic that can be measured. This is the interval between the target presentation and when the eye starts to move in a saccade. To examine individual variability in SRT the standard deviation and coefficient of variation can be calculated. Express saccades have very short reaction times and are generated automatically to
a novel object in the visual scene. These generally occur between 90-120ms after the appearance of the new object. There is a delay of approximately 90ms due to neural processing in the retina, cerebral cortex, superior colliculus (SC), and the efferent motor pathway (Dorris et al. 1997; Pare and Munoz 1996). Express saccades improve with practice suggesting that they may reflect a predictive mechanism (Fischer and Ramsperger 1986).

1.4.2 Neurophysiology

When a saccade is generated, either automatically or voluntarily, it involves the coordination of many brain regions (Fig. 1-1). In the primary visual cortex, the localization of the stimulus of interest is represented by activity in cells with retinotopic visual receptive fields. Neurons are organized into a ‘map’. In other words, this map corresponds to two dimensional location on the retina and is coded based on neuron activity. This input must then be transformed into a motor command in the ocular motoneurons which encode the characteristics of the saccade in terms of temporal discharge, where the size of the saccade is proportional to the total number of discharge spikes (Crawford and Guitton 1997). The motoneurons are innervated by connections in the brainstem. Electrophysiology has revealed that the pons is important for generation of horizontal saccades. Specifically, excitatory neurons in the paramedian pontine reticular formation are essential for driving the initial force to generate a horizontal saccade. The rostral mesencephalon is important for the generation of vertical saccades. Specifically, excitatory burst neurons in the rostral interstitial nucleus of the medial longitudinal fasciculus are essential for the initial velocity. There are also inhibitory burst neurons which act to silence the antagonistic oculomotor muscles during the saccade (Henn et al. 1989). Long lead burst neurons are located in the brainstem reticular formation and project to the excitatory and inhibitory burst neurons causing them to discharge (Scudder et al. 1996). Finally, omnipause neurons located in
the nucleus raphe interpositus inhibit burst cells to keep the eyes stationary and therefore discharge continuously except during a saccade (Buttner-Ennever et al. 1988). Brainstem structures are also involved in shaping the motor output through connections with the cerebellum.
Figure 1-1 The circuitry involved in eye movement control. Modified from Munoz et. al. 2007. SEF=suplémentary eye fields; DLPFC= dorsolateral prefrontal cortex; FEF= frontal eye fields; LGN; lateral geniculate nucleus; PPRF= paramedian pontine reticular formation.
The nucleus reticularis tegmenti pontis encodes the size and direction of saccades in three dimensional eye displacement vectors (Van et al. 1996). It projects to the dorsal vermis and caudal fastigial nucleus of the cerebellum. The dorsal vermis is involved in modulating on-line amplitude and trajectory during a saccade (Keller et al. 1983). The fastigial nucleus works with the dorsal vermis to control saccade accuracy by monitoring motor commands via a feedback model in which internal feedback of a motor command (effference copy) corrects for anticipated errors by rapid modifications of saccade duration (Robinson and Fuchs 2001). Therefore, these interconnected structures play a role in saccade metrics and accuracy.

Another connection to the brainstem comes from the SC. The SC consists of seven layers with the dorsal layers primarily involved in vision and the ventral layers primarily involved in motor commands (Moschovakis et al. 1988a;Moschovakis et al. 1988b). Therefore, the dorsal layers contain a map of retinal projections of the visual field, while the ventral layers contain a motor map of eye movements. Electrical stimulation to the ventral layers of the SC encodes the direction and size of the saccade depending on the site of stimulation (Robinson 1972). Within the ventral layers, stimulation to caudal regions lead to larger saccades and rostral regions lead to smaller saccades. Similarly, stimulation to the medial or lateral regions leads to upward saccades or downward saccades, respectively. The saccades occur in an all-or-none fashion once a certain threshold of activity has been reached (Leigh & Zee 2006). The ventral SC receives connections from the frontal and parietal lobes, where the dorsal SC receives connections from the retina and occipital cortex.

Two cortical regions important for control of eye movements include the frontal and parietal lobes. The frontal lobe houses the frontal eye fields (FEF), supplementary eye fields (SEF) and dorsolateral prefrontal cortex (dLPFC). Like the ventral layers of the SC, the FEF are
also involved in saccade production. Microstimulation to the FEF in the rhesus monkey elicits a saccade of a specific direction and amplitude (Bruce et al. 1985). The SEF contain neurons involved in voluntary saccades and are involved in learned, complex eye movements (Everling et al. 1998b; Petit et al. 1996). The dIPFC is also involved in voluntary saccades especially those that require inhibition and memory (Sweeney et al. 1996). The parietal cortex houses the posterior parietal and parietal eye fields. The posterior parietal cortex is important for shifts of visual attention to objects of interest. The parietal eye fields are thought to be primarily involved with making saccades to specified targets in spatial locations (Thier and Andersen 1998). The frontal lobe has reciprocal connections with the parietal lobe and is innervated by the thalamus.

Two parts of the thalamus contribute to saccades including the pulvinar and cerebral nuclei of the internal medullary lamina (IML). Neurons in the pulvinar appear to play a role in retinal image motion and shifting visual attention (LaBerge and Buchsbaum 1990; Robinson et al. 1991). Neurons in the IML are involved in both voluntary and visually-guided saccades. It is thought that the IML may be a source of efference copy information to the cortical eye fields because it receives inputs from cortical and SC structures involved in eye movements but projects only to the frontal lobe and caudate nucleus (CN) of the basal ganglia (Schlag-Rey and Schlag 1984). The CN also receives projections from the frontal lobe structures involved in eye movements, as well as reciprocal connections with other basal ganglia structures such as the substantia nigra pars compacta (SNp). The CN is involved in more complex behaviours involved in regulating eye movements such as memory, expectations, attention, and reward (Hikosaka et al. 1989; Kato et al. 1995). The SNp plays a role in visually-guided or memory-guided, voluntary saccades (Hikosaka and Wurtz 1983a; Hikosaka and Wurtz 1983b; Hikosaka and Wurtz 1983c).
1.4.3 Eye Movement Experimental Measures

Many different tasks exist to measure various aspects of the eye movement system. The smooth pursuit system can be measured by requiring participants to follow or track a moving object. The visual fixation system is measured by requiring participants to fixate on a stationary target. Combined head and eye movements can also be examined by requiring participants to make rapid gaze changes which involve the use of both their head and eyes to look to a particular target of interest. Vergence eye movements can be measured by eliciting disparity between the location of images on the retina of each eye (fusional vergence), or by stimulating a loss of focus on the retina (accommodative vergence). Finally, the saccadic system can be measured by instructing participants to make automatic or voluntary saccades. For the current thesis the saccadic system was measured by using the prosaccade, antisaccade, and memory-guided eye movement tasks. These tasks were chosen based on maintaining an efficient but exhaustive protocol so a variety of measures could be obtained. From these three tasks several basic sensory-motor, working memory, inhibition, and visuospatial processing measures were acquired. These measures are important to measure in children with FASD because these are domains of function found to be affected by prenatal alcohol exposure using psychometric testing.

1.4.3.1 Prosaccade task

The prosaccade task is a visually-guided saccade task that measures basic sensory-motor integration (Fig. 1-2A). The task requires the participant to fixate on a central fixation point (FP) and look to a peripheral target when it appears. This task has three variations. The first is a gap task in which the FP disappears approximately 200ms before the appearance of a peripheral visual stimulus. The second is a step task in which the peripheral target appears simultaneously
with the disappearance of the FP. The third is an overlap task in which the FP remains visible while the peripheral target appears. In the current thesis the gap task was used with a 200ms delay and peripheral target that appeared 10 degrees to the left or right of the FP. The gap condition causes reduced reaction time and increased frequency of express saccades compared to the overlap condition (Fischer and Ramsperger 1984; Saslow 1967). Neurons found in the SC exhibit low frequency activity that gradually increases about 100ms after the beginning of the gap period and is negatively correlated with SRT (Dorris, Pare, & Munoz 1997). This indicates that the higher the level of activity in these SC neurons the faster the reaction times because these neurons are closer to saccade initiation threshold. Additionally, similar patterns of low frequency buildup during the gap period have been found in the FEF, dIPFC, and lateral intraparietal cortex (LIP) (Ben and Duhamel 2002; Everling and Munoz 2000; Tinsley and Everling 2002).
Figure 1-2 Eye movement tasks. A: Prosaccade Task: Correct fixations (circles) and saccades (arrows). B: Antisaccade Task: Correct fixations (circles) and saccades (arrows). C: Memory-Guided Saccade Task: Correct fixations (circles) and saccades (arrows). Grid is not visible to participant.
1.4.3.2 Antisaccade task

The antisaccade task is a voluntary saccade task that measures inhibitory skills by requiring the suppression of an automatic saccade in favor of a voluntary saccade (Fig. 1-2B). The task requires participants to fixate on a central FP and when a target flashes in the periphery to look towards the opposite side of the screen. In the current thesis, the peripheral target appeared 10 degrees to the left or right of the FP and the participant was instructed to look to the opposite side of the screen. A direction error occurred if the participant looked toward the peripheral target (i.e. made a prosaccade). Electrophysiology studies in monkeys performing the antisaccade task have found that in the SC, fixation related neurons exhibit an increased level of activity and saccade related neurons reduce their low frequency activity compared to prosaccade trials (Everling et al. 1999). When a direction error is made, there is greater low frequency preparatory activity that resembles what occurs during a prosaccade trial (Everling et al. 1998a). The FEF show patterns similar to the SC with lower preparatory activity (Everling & Munoz 2000). The dIPFC shows task selectivity when comparing prosaccade trials to antisaccade trials in a top-down fashion (DeSouza et al. 2003;Johnston et al. 2007). Another source of top down volitional control comes from the SEF, as many SEF neurons exhibit higher activity for antisaccades compared to prosaccades (Schlag-Rey et al. 1997). Finally, like in the prosaccade task area LIP is also involved and shows increased stimulus related response on antisaccade trials and also seems to be involved in the remapping of the vector signal (Gottlieb and Goldberg 1999;Zhang and Barash 2000).

1.4.3.3 Memory-guided task

The memory-guided saccade task is a voluntary saccade task that measures working memory and response inhibition (Fig. 1-2C). The task requires participants to fixate on a central
FP for as long it is appears on the screen. While the participant is fixating on the FP a visual stimulus appears in the periphery at one of several possible locations. The participant is required to wait for the FP to disappear and is instructed to make a saccade to the remembered location(s). If two or more stimuli are flashed then the participant is also instructed to remember in what order the targets flashed. In the current thesis, two visual stimuli were flashed in sequence at two different locations of 36 possible locations. The delay between the stimuli appearance and the FP disappearance varied randomly for 0, 600, 1200, or 1800ms. A timing error occurs if the participant looks to the target locations before the FP disappears. A sequence error occurs if the participant makes an eye movement to the two target locations in the wrong order. The dLPFC has consistently been shown to play a large role in successful completion of this task. These neurons have been found to have increased activity during the delay period indicating it may be a neural correlate of short term memory (Fuster and Alexander 1971). Additionally prefrontal neurons also show fine tuning to spatial locations and the area to which a neuron responds best has been referred to as the memory field (Funahashi et al. 1989). The dLPFC has also been shown to send stimulus, delay, and saccade related activity to the SC (Johnston, Levin, Koval, & Everling 2007). Area LIP has also been found to show activity similar to the dLPFC as they respond in a time locked fashion to visual stimuli, delay periods, and saccades (Colby et al. 1996; Gnadt and Andersen 1988). Area LIP has also been found to send connections to the SC in a similar fashion to the dLPFC (Pare and Wurtz 1997). In the FEF most presaccadic cells are tuned to the dimensions of saccadic eye movements rather than to the coordinates of retinal stimulation and also show delay and saccade related activity (Goldberg and Bruce 1990). Like the dLPFC and the LIP, the FEF convey delay activity related to movement, memory, and vision.
to the SC (Sommer and Wurtz 2001). What differentiates the FEF from the dIPFC and LIP is that the activity of these neurons is correlated with SRT (Everling & Munoz 2000).

**1.4.4 Eye Movement Control and FASD**

The first eye movement control study in FASD was published in 2007. In a small sample (n=10) of children with FASD (8-12 years old), Green et al. (2007) examined gap and overlap conditions for the prosaccade and antisaccade tasks using electrooculography. The authors found an increased SRT and decreased percentage of express saccades in the children with FASD compared with typically developing children, in both the gap and overlap conditions of the prosaccade task and an increased percentage of direction errors selectively in the overlap condition. On the antisaccade task only an increased SRT was found for both the gap and overlap tasks (Green et al. 2007). The same research group again examined the prosaccade and antisaccade tasks in a larger group of children with FASD (8-15 years old; n=89) and were able to divide the FASD group into diagnostic subgroups for individual analysis. This study utilized the ISCAN (ISCAN Inc., Burlington, MA, USA), which required the children to wear a head-mounted infrared camera. On the prosaccade task, the ARND and FAS groups, but not the pFAS group, were found to have significantly increased SRT compared to the controls. Additionally, the pFAS and FAS groups were found to have increased frequency of direction errors when compared to controls on the prosaccade task. On the antisaccade task, the pFAS and FAS groups were found to have significantly increased SRT compared to both the control and the ARND groups. All three diagnostic subgroups had increased percentage of direction errors compared to the age matched controls, with the pFAS and FAS groups significantly different from the ARND group (Green et al. 2009a).
In 2013, Tseng et al. published a paper from a novel free viewing task administered using the ISCAN. This study was completed in children with FASD (mean age 12.3; n=13) with a task which required participants to simply watch 15 minutes of random short video clips with no further instruction. Two hundred and twenty four quantitative features of saccades were then extracted and run through a microarray analysis to identify critical features that differentiated participants with FASD from controls. The analysis was able to classify FASD versus controls at 79.2% accuracy and FASD versus ADHD at 90.4% accuracy. Interestingly, the classifier was more likely to misplace a child with FASD in the control group than the ADHD group (Tseng et al. 2013a). This study was repeated recently using the Eyelink 1000 (SR Research, Mississauga, ON, Canada), a device which uses a remotely mounted infrared light and camera with nothing touching the child, allowing for more natural viewing behavior. In a cohort of children with FASD (5-18 years old; n=47), using only 5 minutes of video clips, the classification accuracy obtained was 74.1% in FASD children versus control children. Although this accuracy was not improved, the first paper included analysis based only on saccades while the new analysis contains additional eye movement features such as gaze (Tseng et al. 2013b).

Coffman et al. (2013) published a paper that paired prosaccade measures obtained using the Eyelink 1000 with magnetoencephalography (MEG) in a small group of adolescents with FASD (12-21 years old; n=18). The authors found that the latency of the FASD group’s primary visual M100 response in V1 was delayed for both the central and peripheral target stimuli. This indicates an overall visual system impairment in the FASD group. The authors also found that when the difference in latency of the M100 response to central vs. peripheral targets was examined it was only different in the FASD group. This indicates that the visual system impairment is most pronounced for the peripheral targets. However, in contrast to Green et al.
(2007;2009) the authors did not find a difference in SRT (Coffman et al. 2013). This may be due to the difference in equipment used to record the eye movement data (i.e. ISCAN vs. Eyelink 1000), since the ISCAN is a head-mounted device and the Eyelink 1000 is remotely mounted. This may also be due to the differences in age as the Green et al. (2007;2009) study was conducted in mainly children whereas this study mainly used adolescents. In the same dataset, this research group also found four clusters over the right frontal and parietal cortex and left temporal/occipital cortex in the FASD group that significantly differed from controls for left target presentation. Interestingly, the authors did not find any differences for right target presentation (Stephen et al. 2013).

Finally in 2014, Hemington and Reynolds paired a single channel electroencephalographic (EEG) device with a memory-guided eye movement task in children with FASD (8-18 years old; n=18) using the Eyelink 1000. This task required children to remember the spatial location of one, two, or three targets while wearing a single electrode EEG headband. The children with FASD made more timing errors on the two and three, but not one, target. This indicates that when the cognitive load is increased, children with FASD show greater deficits compared to controls. Additionally, the errors correlated to reductions in alpha and theta power selectively in the FASD group. Taken together, these data indicate that more cognitive resources are required for children with FASD to complete the task and this may indicate differing patterns of neural recruitment (Hemington and Reynolds 2014).
1.5 Diffusion Tensor Imaging (DTI)

1.5.1 Diffusion Tensor Imaging Background

The current thesis employed a specialized MRI technique called DTI. Before DTI, structural magnetic MRI was being used to study the gray and white matter, as well as the cerebrospinal fluid within the brain. This was accomplished by using the contrast between tissue types to allow the size and shape of the whole brain and its structures to be quantified. Gross anatomy was then characterized in the healthy or diseased brain, as well as throughout development (Cascio et al. 2007). Functional MRI (fMRI) stemmed from structural imaging to allow researchers to examine the areas of the brain active during performance of a particular task, at rest, following presentation of a stimulus, or during cognitive processing, etc. The basic method involves imaging of the brain or spinal cord repeatedly for several minutes to detect changes in the images over time. The MRI signal can be made to vary with changes in neuronal activity by measuring the blood oxygenation-level dependent contrast in tissue. Researchers can then infer that any regions of the CNS that changed in the images between two states involve that region (Stroman 2011). Finally, diffusion-weighted imaging was developed to detect changes in tissue structure at a cellular level using activity-dependent changes in water self-diffusion by examining the apparent water diffusion coefficients which are strongly influenced by cellular structures that restrict water movement. This can then reveal details about tissue structure at the cellular level such as orientation of axons in white matter (Stroman 2011). DTI is an application of this method and was chosen for this thesis to better investigate white matter abnormalities previously found in children with FASD and relate these to the functional outcome measures.
The dominant source of signal from all MRI techniques comes from the nucleus of hydrogen atoms. Since hydrogen is found in water and lipids it is the ideal target for imaging biological tissues as both are abundant in the brain. When placed in a strong magnetic field the hydrogen molecules are forced into alignment with that field but when a radio frequency pulse is added it causes the hydrogen atoms to become out of phase. As the hydrogen atoms return back to alignment (relaxation), the receiver coil of the MRI system detects this signal. Since different tissues (i.e. grey matter versus white matter) have different relaxation times the strength of the signal from this can be used to differentiate tissue types. Spatial information is encoded by applying additional magnetic fields (gradients) to cause the signal to vary with position.

DTI was developed in the mid-1980s as a means of better characterizing white matter in the central nervous system noninvasively, and to provide additional and more accurate information than the current structural imaging available (Basser et al. 1994). This was accomplished by sensitizing the MRI signal to the thermally-driven, random molecular motion of water molecules (Brownian motion) via a diffusion-encoding gradient (Jones and Leemans 2011). This allowed the rate at which the water molecules diffuse to be measured and the underlying neuronal mechanisms to be studied. DTI involves the application of a gradient in one direction and then measuring diffusion in that direction. In a diffusion-weighted sequence, a strong gradient is applied in one direction, after a 90° radio-frequency pulse, which causes the water protons to become out of phase. The second gradient is opposite and therefore causes the effects to be cancelled out in stationary tissue. However, if diffusion occurs then the second gradient causes the protons to be in a different spatial position along the gradient (Bosma and Stroman 2012). Therefore, the measurement of the signal intensity can be used to determine the apparent diffusion coefficient in the direction of the applied gradient. The diffusion coefficient is
considered ‘apparent’ because other processes such as pressure gradients, temperature, ion-ion interactions, or membrane permeability occur along with the diffusion properties resulting from anisotropy (Bosma & Stroman 2012).

Compared to other imaging techniques, DTI provides a superior method to estimate the different paths water takes within the white matter as it diffuses to different regions. In an isotropic environment, molecules that are unrestricted are able to move equally in all directions. For example, the water molecules in the cerebrospinal fluid can move freely within the ventricles (Fig. 1-3). However, in an anisotropic environment, such as tissues in the brain, the orientation of diffusion becomes restricted and differentially moves in one direction more than any other (Moseley et al. 1990). In fiber bundles in the brain, the diffusion occurs more readily in the direction parallel to the axons rather than perpendicular (Fig. 1-3). This is due to the structure and insulation properties of myelin which allows it to highly restrict water movement (Beaulieu 2002). By using these principles of diffusion, data can be acquired regarding the orientation and magnitude of water movement within the brain.

DTI measures magnitude and orientation of water diffusion in three-dimensions and subsequently allows for mapping of white matter structure by reconstructing the data into a three-dimensional volume via multiple diffusion encoding directions. This estimates the diffusion tensor (Basser, Mattiello, & LeBihan 1994). This method also allows for measurement of tissue integrity on a voxel-by-voxel level (Wozniak and Muetzel 2011). At least six different gradient directions are required to adequately characterize a diffusion tensor. Additionally, one non-diffusion weighted image, also known as b0, is required as a baseline. Following data acquisition, a diffusion tensor matrix is constructed and diagonalization of the tensor is used to calculate the eigenvectors and eigenvalues (Basser and Pierpaoli 1998).
Figure 1-3  

**Isotropic Diffusion:** When water molecules are in an isotropic environment, the motion occurs equally and randomly in all directions (ex. the ventricles). When water molecules are in an anisotropic environment, the motion is oriented more in one direction than any other (ex. white matter).
1.5.2 DTI Measures

The largest eigenvalue ($\lambda_1$) is in the primary diffusion direction, the second largest eigenvalue ($\lambda_2$) is in the diffusion direction orthogonal to the first, and the smallest eigenvalue ($\lambda_3$) is in the final orthogonal direction. The sum of these three eigenvalues is known as the ‘trace’ of the diffusion tensor and the average of the values is known as the mean diffusivity (MD). Gray matter and white matter tend to have lower MDs while cerebrospinal fluid, which appears brightly on MD maps, has a much higher value. In white matter, $\lambda_1$ represents the diffusivity parallel to the axons and is known as parallel or axial diffusivity. The $\lambda_2$ and $\lambda_3$ are usually averaged, as they are both orthogonal to the axons, and are known as radial or perpendicular diffusivity (Basser 1995). Finally, the most commonly reported measure is fractional anisotropy (FA), which relates to the ratio of diffusion in different directions (Masutani et al. 2003). FA ranges from zero, in an isotropic environment, to 1 in a highly anisotropic environment (Paydar et al. 2013). FA is much more variable across the brain than MD, with higher values for white matter and lower values for gray matter. The lowest FA values can be found in the cerebrospinal fluid. The diffusion ellipsoids reflect the three orthogonal eigenvalues and therefore also the FA and MD (Bosma & Stroman 2012). As FA increases and MD decreases, the ellipsoids become more elongated. For example, the cerebrospinal fluid has a high MD and a low FA with an ellipsoid near spherical, whereas the corpus callosum has a higher FA and a lower MD with an elongated ellipsoid. DTI can therefore be used effectively to provide different measures with relatively high sensitivity when characterizing white matter.

The DTI measures stated previously do not provide a visual measure that can be used by researchers to understand their data. Therefore, FA colour maps may provide an additional way of looking at DTI data. An FA map uses colour to indicate primary diffusion direction in each
voxel; wherein green represents anterior-posterior, blue represents inferior-superior, and red represents left-right. FA maps also display FA values via intensity variation which allows one to focus more on the white matter, which appears brightly, and less on gray matter and cerebrospinal fluid which appear darker. One advantage of the FA map is that the information added by the colour allows a researcher to perform quality checks based on known white matter tract characteristics. For example, if a colour map displays the corpus callosum as green, then the researcher can identify that something is erroneous with the data and can perform the proper follow-up. Another advantage is that colour maps allow for easy delineation of structures with similar FA, but different orientation such as the anterior and posterior limbs of the internal capsule.

1.5.3 DTI Analysis

There are three different, but commonly used analysis techniques that can be applied to DTI data. The first is region of interest (ROI) analysis, which involves the placement of two-dimensional regions on a single DTI image. This can be used to analyze several well defined brain structures such as the corpus callosum, anterior and posterior limbs of the internal capsule, thalamus, etc. ROI analysis is usually hand drawn regions on a single slice of a FA map, MD map, colour map, or b0 image. The map/image chosen is usually dependent on where the structure of interest is most easily defined from surrounding structures. DTI variables, like FA and MD, are then calculated for each voxel and the final output is the average of all voxels in the ROI (Niogi et al. 2007). The major advantage of ROI analysis is that it is useful when assessing subsections of structures and requires less processing since the images do not need to be normalized. A disadvantage is that it requires each ROI to be manually drawn, which can be time consuming. Since there is individual variability in manual drawings, to maintain consistency the
same rater should perform the analysis on each participant. Thus, this technique is sometimes useful and simple to learn, but not appropriate for all DTI analyses.

The disadvantages of ROI analysis led researchers to investigate new techniques to analyze DTI data. The second technique used by researchers is tractography, which eliminates some of the limitations of manual ROI analysis. Tractography uses the primary diffusion direction and anisotropy to trace the white matter pathway within each voxel. This method is slightly more theoretical, consisting of a sophisticated math-based approach to connect the dots. Deterministic tractography is one of two methods commonly used. It begins with a seed point, that is either manually drawn or automated, and follows the direction of the primary diffusion vector from voxel to voxel until the FA falls below a predetermined angle or threshold (Basser et al. 2000). One advantage of this method is the resulting fiber pathways can be visualized in three-dimensions and many statistical measures can be derived from the entire pathway. The disadvantages associated with deterministic tractography are that the tracts can be lost in areas with crossing fibers and there are no confidence measures given. The other method is probabilistic tractography, which attempts to compensate for these problems by estimating uncertainty values for each voxel and producing multiple pathways (Behrens et al. 2003). Tractography and ROI analysis can be a very useful tool for hypothesis-based analyses; however, it is extremely difficult to use for whole brain analyses.

For whole brain analysis, voxel-based morphometry is much more useful. Briefly, it requires the data to be normalized to a template, followed by smoothing, and finally, analysis of each voxel (Ashburner and Friston 2000). Voxel based analysis has several advantages insofar that it is rater-independent and can detect small areas of group differences or correlations. However, the data must be heavily processed which can alter results, such as FA and MD,
especially when there is increased variation between subjects. Additionally, during the analysis phase, a very strict p-value must be set due to the large number of comparisons being made. Therefore, when analyzing DTI data one must spend a considerable amount of time deciding which analysis tool is best suited for the research question.

1.5.4 DTI and FASD

Before the advent of DTI, many structural MRI studies demonstrated abnormalities in both the gray and white matter regions in FASD participants (Autti-Ramo et al. 2002; Riley et al. 1995; Swayze et al. 1997). These findings of macrostructural aberrations led to the search for more subtle abnormalities. The first DTI study conducted in individuals with FASD examined young adults (18-25 years old; n=9) with FAS, the subtype that presents with facial abnormalities. The authors used ROI analysis on two regions of the corpus callosum and found decreased FA and increased MD in the genu and splenium of the FAS participants compared to the controls. The authors also correlated facial dysmorphology and cognitive scores with these DTI measures but found no significant correlations (Ma et al. 2005).

Similar to Ma et al. (2005), Wozniak et al. (2006) used manual ROIs to analyze the corpus callosum. However, these researchers included participants with mild to moderate FASD and excluded those with FAS (10 to 13 years old; n=14). This paper was also distinct because the authors used children instead of young adult participants and six regions of interest in the corpus callosum instead of two. This study analyzed FA and MD measures and found a significantly greater MD in the isthmus of children with FASD. Similar to the previous study, the authors also attempted to correlate facial dysmorphology scores with the DTI measures and did not find any significant relationship (Wozniak et al. 2006). This is not surprising as these children did not have FAS and therefore would have few to no facial features present.
Sowell et al. (2008a) was the third DTI study to be published, but the first to use voxel-based analysis in children with FASD (7-15 years old; n=17). The paper reported lower FA in the right lateral temporal lobe, bilaterally in the splenium, bilateral posterior cingulate, right internal capsule, and brainstem in the FASD group compared to the control group. A *post hoc* FA white matter ROI analysis was then performed to confirm the FA differences were indeed in white matter. The DTI scores were also correlated to cognitive tests and a positive relationship was observed in the FASD group for splenium FA and visuomotor integration. No correlations were observed in the control group (Sowell et al. 2008a).

Lebel et al. (2008) was the first to use semi-automated tractography to delineate ten major white matter tracts in children with FASD (5-13 years old; n=24). The authors also performed ROI analysis of four deep gray matter structures. Tractography analysis revealed that the FASD group had significantly decreased FA in the splenium, right cingulum, bilateral inferior longitudinal fasciculus, and bilateral superior longitudinal fasciculus. ROI analysis revealed an increased FA in the bilateral globus pallidus and decreased FA in the left thalamus in the FASD group compared to the controls. Interestingly, there was very little overlap between FA and MD results and the FASD group was found to have significantly greater MD in the right corticospinal, bilateral inferior frontooccipital fasciculus, left inferior longitudinal fasciculus, bilateral globus pallidus, right putamen, and right thalamus. The MD of the genu was found to be significantly decreased in the FASD group which directly contrasts the Ma et al. (2005) findings. The findings of increased FA and decreased MD in some regions of the FASD group are counterintuitive to the previous findings which hypothesize that higher FA and lower MD are indicative of a well-organized, higher functioning brain. The authors postulate that these findings could be due to damage to regions of crossed fibers. This group also performed correlational
analysis with these DTI measures and cognitive tests but found no significant results (Lebel et al. 2008).

The fifth DTI paper on FASD to be published was by Fryer et al. (2009) which used voxel based analysis in children with FASD (8-18 years old; n=15). They found significantly lower FA in the FASD group in several regions of white matter including the right superior longitudinal fasciculus, bilateral corona radiata, bilateral forceps major, bilateral sagittal stratum, right uncinate fasciculus, and the body of the corpus callosum. Higher FA was found in the FASD group when compared to controls in the right internal capsule and right cingulum. In contrast to other studies, there were no group differences found for MD (Fryer et al. 2009). The results in this study have very little overlap with the other voxel based analysis by Sowell et al. (2008a). This could be because Fryer et al. (2009) used tract-based spatial statistics (TBSS), which focuses on central white matter voxels in order to minimize the effect of inter-subject registration problems. This method may be more sensitive to the subtle effects of prenatal alcohol exposure on the brain.

In another study which used TBSS to analyze the corpus callosum in young adults with FASD (19-27 years old; n=57), Li et al. (2009) reported lower FA in the isthmus of the FASD group. This was the only FA difference and no other MD, axial diffusivity or radial diffusivity differences were found. This differs from the previous study that found a difference in the FA in the body of the corpus callosum. This may be due to a difference in population age (i.e. Fryer and colleagues used children and Li and colleagues used young adults). A better comparison may be to the Ma et al. (2005) study which also found significantly lower FA in the posterior portion of the corpus callosum in young adults with FAS; however these authors also found differences
in the genu. DTI metrics were correlated with IQ and facial dysmorphology but no significant relationships were found (Li et al. 2009).

Similar to Lebel et al. (2008), Wozniak et al. (2009) used semi-automated tractography to examine six regions of the corpus callosum in children with FASD (10-17 years old; n=33). This was an extension to the research group’s previous work in which they examined the same six regions using manual ROI analysis. The results exhibited a significantly lower FA in the posterior portion of the corpus callosum (posterior midbody, isthmus and splenium) in the FASD group. This result is similar to Lebel et al. (2008) who also found a decreased FA in the splenium and not the body, but differences in the isthmus and posterior midbody may have been lost due to averaging across the entire body. There was a prominent relationship identified in the FASD group between FA in the genu and MD in the splenium to a working memory cognitive task. There was also a significant correlation between splenium MD and a perceptual organization task in the FASD group. As with all the other studies, there was no significant relationship found with facial dysmorphology scores and DTI measures (Wozniak et al. 2009).

Lebel et al. (2010) performed a correlation of voxel based clusters and math scores of children with FASD (5-13 years old; n=21), with no control group. The authors found four significant clusters with correlations between FA and math scores on a cognitive test. The left cerebellum, left parietal lobe, and upper left parietal lobe were all positively correlated to math score. There was one cluster in the bilateral brainstem that was found to be negatively correlated with math scores. However, this counterintuitive finding may have been due to changes in crossing fibers. The authors then performed manual tractography through the significant clusters to identify specific white matter tracts that passed through. The tracts identified include the left
superior longitudinal fasciculus, left corticospinal, body of the corpus callosum, middle cerebellar peduncle, and the internal capsule (Lebel et al. 2010).

The papers that emerged later focused on functional correlations to these DTI findings. Spottiswoode et al. (2011) used voxel based analysis to examine the cerebellum of children with FASD (9-13 years old; n=13). The FASD group had significantly lower FA and higher perpendicular diffusivity in the left middle cerebellar peduncle. Furthermore, there was a positive relationship between the FA in the left middle cerebellar peduncle and performance on eyeblink conditioning (Spottiswoode et al. 2011). As previously mentioned, Lebel et al. (2010) found a positive relationship to math score in this region as well. Green et al. (2013) also performed a correlation with voxel based clusters and eye movement scores of children with FASD (8-13 years old; n=14), with no control group. A positive relationship was found between FA of the anterior and posterior corpus callosum and antisaccade SRT, as well as prosaccade SRT and FA of the genu. A positive relationship was also found between prosaccade SRT and the FA of the right inferior longitudinal fasciculus. Conversely, a negative relationship was found between the prosaccade SRT and the FA of the left cerebellum (Green et al. 2013).

Most recently, Treit et al. (2013) published a study on the developmental trajectories of DTI measures in children with FASD (5-15 years old; n=17). In both groups, FA increased with age and MD decreased with age. Interestingly, the authors found altered developmental progression by observing a greater decrease in MD with age in several tracts in the FASD group. Additionally, the change in MD with age was shown to negatively correlate with reading and receptive vocabulary in the FASD group. Interestingly, there were steeper decreases in MD in the superior fronto-occipital fasciculus and superior longitudinal fasciculus between scans correlating with greater improvement in language scores in the FASD group (Treit et al. 2013).
Although these findings are somewhat consistent, there are some direct contradictions which indicate the need for further investigation of DTI. This is evidenced by the fact that even studies with the same analysis techniques do not find consistent results. These inconsistencies may be due to differences in age, diagnosis, disease severity, sample size, or methodology (different scanners, number of directions, etc.). What is clear is that the most reliable white matter abnormality in FASD is in the corpus callosum, especially in the posterior region. As research moves forward, it will be important to understand the effects that development, as well as alcohol timing and exposure level, have on DTI metrics. This may help solve some of the discrepancies in the research findings and provide better insight into the damage prenatal alcohol exposure causes on white matter as well as establishing effective interventions to mitigate some of these deficits.
1.6 Thesis Rationale, Hypothesis and Objectives

This thesis exploits the involvement of multiple centers across Canada and the results reflect the participation of over 200 volunteer participants, families, caregivers and support workers. There is a clear need to develop new tools for assessing brain function in the FASD population. The diagnostic process for these children is both cumbersome and exhausting, requiring up to 2 days of testing and involving multiple areas of expertise. Thus, faster and more reliable procedures are needed as tools to (i) screen for brain injury and (ii) aid in the diagnostic process. The measurement of eye movement behaviours is an excellent candidate for this, as these measures can be obtained relatively quickly, and are non-invasive and objective. To test the utility of eye tracking in the assessment of brain injury in FASD it needs to be compared to the current gold standard of assessment which is psychometric testing. To do so one must directly compare eye movement scores to psychometric test scores which has never been completed in children with FASD.

Therefore, the overarching hypothesis for this doctoral thesis research was that deficits in eye movement control are correlated to specific psychometric tests and structural abnormalities in the brain of children with FASD. Based on the current state of knowledge and preliminary data analysis the specific hypotheses to be tested were:

(i) Children with FASD will exhibit deficits on behavioural and metric measures of eye movement control;

(ii) The psychometric tests used during the diagnostic process will correlate to eye movement measures that assess the same cognitive domains; and

(iii) The integrity of white matter tracts in the corpus callosum will correlate to deficits in the performance of structured eye movement tasks.
The objectives of this thesis were to:

(i) collect imaging, psychometric and eye tracking data in over 100 FASD and over 100 typically developing children between the ages of 5-18;

(ii) further characterize oculomotor behaviours of children with FASD;

(iii) compare the sensitivity of standardized psychometric tests with measures of saccadic eye movement control to identify brain dysfunction in children with FASD; and

(iv) correlate measures of white matter integrity, as measured using diffusion tensor imaging, to the performance of eye movement tasks.
Chapter 2

Altered accuracy of saccadic eye movements in children with Fetal Alcohol Spectrum Disorder

2.1 Abstract

Background: Prenatal exposure to alcohol is a major, preventable cause of neurobehavioral dysfunction in children worldwide. The measurement and quantification of saccadic eye movements are a powerful tool for assessing sensory, motor, and cognitive function. The quality of the motor process of an eye movement is known as saccade metrics. Saccade accuracy is one component of metrics, which to function optimally requires several cortical brain structures as well as an intact cerebellum and brain stem. The cerebellum has frequently been reported to be damaged by prenatal alcohol exposure. This study, therefore, tested the hypothesis that children with FASD will exhibit deficits in the accuracy of saccades.

Methods: A group of children with FASD (n=27) between the ages of 8 and 16 and typically developing control children (n=27) matched for age and sex, completed three saccadic eye movement tasks of increasing difficulty. Eye movement performance during the tasks was captured using an infrared eye tracker. Saccade metrics (e.g., velocity, amplitude, accuracy) were quantified and compared between the two groups for the three different tasks.

Results: Children with FASD were more variable in saccade endpoint accuracy, which was reflected by statistically significant increases in the error of the initial saccade endpoint and the frequency of additional, corrective saccades required to achieve final fixation. This increased variability in accuracy was amplified when the cognitive demand of the tasks increased. Children with FASD also displayed a statistically significant increase in response inhibition errors.
Conclusions: These data suggest that children with FASD may have deficits in eye movement control and sensory-motor integration including cerebellar circuits, thereby impairing saccade accuracy.
2.2 Introduction

Prenatal exposure to alcohol is a major, preventable cause of neurobehavioral dysfunction in children. The full spectrum of adverse effects induced by prenatal alcohol exposure, which includes several diagnostic subgroups, is collectively referred to as Fetal Alcohol Spectrum Disorder (FASD; Chudley et al., 2005). Children with FASD often present with deficits in executive functions (i.e., response inhibition, planning, and cognitive flexibility), attention, and working memory (Kodituwakku et al., 2009; Mattson et al., 1998; Rasmussen et al., 2005). These deficits contribute to the negative behavioral, neuropsychiatric, and maladaptive secondary disabilities commonly observed in this population (Rasmussen et al., 2008). Previous studies (Green et al., 2007; 2009) have established that eye movement tasks can be used to characterize deficits in executive function and motor control in children with FASD.

The circuitry responsible for the efficient and accurate execution of saccadic eye movements involves multiple cortical and subcortical brain regions, and the roles that these brain regions play in controlling eye movement behaviors have been extensively investigated (Leigh and Zee, 2006; Munoz and Everling, 2004; Scudder et al., 2002 for review). Brain disorders that affect one or more specific areas of the brain may be characterized by a predictable pattern of deficits in eye movement control. The measurement of saccadic eye movements is a powerful tool for assessing damage to the CNS, which may include deficits in the efficiency, performance or quality of the motor process (i.e., metrics). Therefore, analysis of the metrics of eye movement behavior can also be used to assess the contribution of multiple brain structures to oculomotor control (Leigh and Zee, 2006). The current study sought to examine the accuracy component of saccade metrics in children with FASD.
Prenatal alcohol exposure is known to cause structural alterations in the brain. The most widely reported abnormalities from structural magnetic resonance (MR) imaging studies include microcephaly, corpus callosum and cerebellar abnormalities, and reduced volume in the basal ganglia (Mattson et al., 1996; Riley et al., 1995; Sowell et al., 1996). Children with FASD also have difficulties with fine and gross motor skills which are thought to reflect cerebellar and brainstem damage (Blackburn and Whitehurst, 2010). The cerebellar vermis has abnormalities in size and location in FASD (O’Hare et al., 2005). In monkeys, reversible lesions to the cerebellar vermis are known to produce deficits in online correction of saccade trajectory (Keller et al., 1983). The fastigial nucleus is also associated with modulation of saccade trajectory, and inactivation results in ipsilateral and contralateral perturbations of saccade trajectory (Goffart et al., 2004). Together, the cerebellar vermis and the fastigial nucleus play an important role in saccade accuracy (Robinson and Fuchs, 2001). Based on these previous findings, the current study tested the hypothesis that children with FASD will exhibit deficits in the accuracy of saccades to visual targets. Moreover, we additionally predicted that increasing the cognitive demand of the task will exacerbate deficits in saccade accuracy as more brain circuits, that are also potentially impaired, are recruited to complete the task.
2.3 Methods

2.3.1 Participants

All experimental procedures were reviewed and approved by the Human Research Ethics Board at Queen’s University. Children with FASD (average age 12±1 years) were recruited through referrals from clinicians in multidisciplinary diagnostic clinics across Canada and were assessed according to the Canadian Guidelines (Chuldey et al., 2005). The FASD group consisted of 13 males and 14 females (Table 2-1). Typically developing children (average age 12±1 years) were recruited from the same geographical areas. The control group consisted of 12 males and 15 females. Control participants were excluded if they had any neurological or psychiatric disorder, were taking any psychoactive medication, or had a visual disturbance, other than requiring corrective lenses. Participants were asked to withhold any medications typically taken on the day of the testing session to avoid alterations in eye movements. Participants received snacks (juice and granola bars) during the sessions and were allowed breaks when necessary. Participants received a $10 gift card for the 1-hour session.
<table>
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<td>Other</td>
<td>2 (11)</td>
</tr>
</tbody>
</table>

Table 2-1 Descriptive Information for Children in the FASD group
2.3.2 Saccadic eye movement recordings

Participants were seated comfortably in a dark, quiet room on a stable chair. Eye position was recorded using the Eyelink 1000 eye tracking system (SR Research, Mississauga, ON). The 17” LCD monitor and mounted infrared camera were at a distance of 58-64 cm from the left eye. The position of the left pupil was digitized in both the vertical and horizontal axes at a sampling rate of 500 Hz. The performance of each participant was assessed in three tasks, in the following order: prosaccade (Fig. 2-1A), antisaccade (Fig. 2-1B), and memory-guided (Fig. 2-1C). Before each task the eye movements of each participant were calibrated using nine on screen targets (eight around the periphery and one central). The targets were flashed sequentially around the screen and the participant fixated on each one. After calibration, the process was repeated to validate that the average error between fixation and target was less than 2 degrees and that no loss of eye tracking occurred. This also ensured that the participants had no visual disturbances.
Figure 2-1 *Eye movement tasks and measures employed.* A: Prosaccade Task: Correct fixations (circles) and saccades (arrows). B: Antisaccade Task: Correct fixations (circles) and saccades (arrows). C: Memory-Guided Saccade Task: Correct fixations (circles) and saccades (arrows). Grid is not visible to participant. D: Error of saccade trajectory and initial trajectory. The initial trajectory was defined as the angle between the ideal path and the path to 50% of the peak velocity (ψ). The saccade endpoint (θ) was measured as the angle (in degrees) between an ideal path to the target and the trajectory of the first saccade. E: Eye trace of a control 16 year old male (blue, solid line) and of a 15 year old male with FASD (red, dashed line) on a prosaccade trial. F: Velocity profiles of the above eye traces. Control 16 year old male in blue solid line and FASD 15 year old male in red dashed line on a prosaccade trial.
2.3.3 Behavioural Tasks

In both the prosaccade and antisaccade tasks, each trial started with illumination of a central fixation point (FP) for 800-1200ms. The FP then disappeared and, after a delay of 200ms (gap period), a peripheral target appeared randomly at 10° to the left or right of the central FP. Participants were given 1000ms to initiate and complete a saccade to the correct location. In the prosaccade task, participants were instructed to look towards the target as soon as it appeared. In the antisaccade task, participants were instructed to look away from the target, towards the opposite side of the screen. No error feedback was given. One block of 60 prosaccade trials and one block of 60 antisaccade trials were obtained from each participant. After instructions were given, the participant repeated the instructions back to the eye tracking administrator to ensure that they understood the task instructions.

In the memory-guided saccade task, participants were instructed to maintain fixation at the central FP while two peripheral targets appeared. After the FP disappeared, the participants were required to make a saccade to the remembered locations (Fig. 2-1C). The screen was divided into four quadrants in which the peripheral targets could appear. Each quadrant consisted of 9 potential target locations in an invisible 3 by 3 grid centered at a 10° visual angle from the FP. The FP was illuminated for 200-1000ms before the appearance of the two targets. The two targets then appeared briefly in immediate succession for 100ms each, within two of the four quadrants of the screen. Participants were required to fixate for an additional random time of 0, 600, 1200, or 1800ms between the disappearance of the second peripheral target and the disappearance of the FP. The participants were instructed to remember the order and spatial location of the peripheral targets, and to make two saccades as accurately as possible to these locations in the same sequence but only after the disappearance of the FP. A single block of 72
trials was collected for this task. This task measured the ability of participants to maintain accurate memory of two visual targets, thereby assessing saccade accuracy after variable delay and in the absence of sensory feedback. After instructions were given, the participant repeated the instructions back to the eye tracking administrator to ensure that they understood the task instructions.

2.3.4 Data Analysis

Data were analyzed using custom software developed in MATLAB (R2009b, The Mathworks, Inc, Natick, Massachusetts). Saccades were defined as having a speed of greater than 2.5 times the standard deviation of the background noise (measured during fixation) for at least five sample points. Only trials where the participant was fixating on the FP at the appropriate time were used. If the participant broke fixation inappropriately (i.e., not to a target location or away from the screen) the trial was discarded from analysis. Any trials where eye tracking was lost were removed. To be included in the analysis, each participant had to achieve greater than 50% viable trials in each of the tasks. Two children were excluded before analysis because less than 50% of the trials were viable.

Saccade metrics (efficiency, performance or quality of the motor process) for all correct trials for the three tasks were assessed by examining the following outcome measures: amplitude (°); peak eye velocity (°/s); the error in the initial saccade trajectory (ϕ, defined as the angle between the ideal path and the actual path at 50% of the peak velocity, see Fig. 2-1D); the error of the final saccade trajectory (θ, defined as the angle between the ideal path to the target and the trajectory of the first saccade toward the goal drawn as a straight line from the beginning to the end of the saccade, Fig. 2-1D); and the percentage of trials that contained multiple saccades (more than one saccade generated in the direction toward the target, Fig. 2-1E). Saccadic
reaction time (SRT) in the prosaccade and antisaccade tasks was defined as the time from the appearance of the peripheral target to the initiation of the first saccade during a correct trial. In the memory-guided saccade task, SRT of both the first and second saccades were calculated from disappearance of the central FP during a correct trial. In the memory-guided task, the accuracy of the first and final fixation was defined as the distance of the closest fixation to the actual peripheral target location, for all correct trials. Differences between the groups were analyzed for initial saccade trajectory, saccade endpoint and corrective saccades using a 2-way repeated measures analysis of variance (ANOVA) coupled with Bonferroni post-hoc tests for multiple comparisons. The variability in the saccade endpoints in the prosaccade task for each participant was quantified by: collapsing left and right end points, centering to zero, and then computing the principal axes of the data and their respective magnitudes using principal components analysis (PCA). The ellipse was scaled to represent 2 standard deviations of the data and the resulting area was calculated. Effect sizes were also calculated for the dependent variables using Cohen’s $d$ scores (Cohen, 1988). Data are expressed as mean ± s.e.m. for children in the FASD ($n = 27$) and control ($n = 27$) groups, which were matched as closely as possible for age and gender (Table 2-1).

All viable trials were analyzed for anticipatory, direction, timing and sequence errors. Saccades generated less than 90ms after the appropriate go signal were classified as anticipatory saccades (Munoz et al., 1998). Direction errors in the prosaccade and antisaccade tasks were defined as any initial saccade in the wrong direction with respect to the instruction (i.e., away from the target in the prosaccade task; towards the target in the antisaccade task). Individual trials in the memory-guided task were assigned as either correct, timing errors (saccades initiated before 90ms after the go signal), and/or sequence errors (initial saccade made closer to the
second peripheral target location than to the first target). These measures assessed spatial working memory, response inhibition and attention, and were examined because they are frequently reported as abnormal in children with FASD (Kodituwakkul et al., 2009; Mattson et al., 1998; Rasmussen et al., 2005). The SRT, accuracy of both saccades, and direction and timing errors in the memory-guided task were analyzed using an unpaired t-test.
2.4 Results

2.4.1 Corrective Saccades: Prosaccade and Antisaccade Tasks

Two-way repeated measures ANOVA was used to assess differences in the frequency of corrective saccades for the prosaccade and antisaccade tasks, with group (Control, FASD) as the between-subject factor and task (prosaccade, antisaccade) as the within-subject factor. This analysis revealed statistically significant main effects of group ($F(1,52) = 7.26, p=0.0095$) and task ($F(1,52) = 24.15, p<0.0001$), indicating that children with FASD often made more than one saccade to acquire the target (e.g., Fig. 2-1E red dashed trace), and these corrective saccades were made more frequently in the prosaccade compared with the antisaccade task (Fig. 2-2A).
Figure 2-2 Accuracy measures in the prosaccade and antisaccade tasks. Data are mean ± s.e.m. for participants in the FASD (n=27) and control (n=27) groups. A: Percentage of corrective saccades in the prosaccade task for control (open blue) and FASD (open red) groups (left). Prosaccade effect size (d) is -0.65. Percentage of corrective saccades in the antisaccade task for control (solid blue) and FASD (solid red) groups (right). Antisaccade effect size (d) is -0.57. B: Accuracy of the initial trajectory towards the target and accuracy of the initial saccade endpoint towards the target. Solid line indicates control group and dashed line indicates FASD group. Performance on the prosaccade task for the control group is represented by an open blue circle and FASD group by an open red square. Prosaccade effect size (d) is -0.59 for saccade endpoint error. Performance on the antisaccade task for the control group is represented by a solid blue circle and FASD group by a solid red square. Antisaccade effect size (d) is -0.63 for saccade endpoint error. C: The variability in the saccade endpoints in the prosaccade task for each participant quantified by PCA. Effect size (d) is -0.79. Inset: Endpoints of the initial saccade for the prosaccade task in the right direction, where the ellipses represent mean ± 2 standard deviations for each group. Dashed red ellipse and open circles are FASD population and solid blue ellipse and closed circles are control population. Dotted grey shaded circle indicates target. *p<0.05 compared with control participants.
2.4.2 Initial Trajectory and Saccade Endpoint: Prosaccade and Antisaccade Tasks

Differences between the initial saccade trajectory and the saccade endpoint were examined using two-way repeated measures ANOVA with group (Control, FASD) as the between-subject factor and error (initial saccade trajectory (ϕ), initial saccade endpoint (θ)) as the within-subject factor. For the prosaccade task there was a significant main effect of error ($F(1,52) = 63.85, p<0.0001$) but not group ($p>0.05$), indicating that children in both the control and FASD groups improved their accuracy from the initial trajectory (ϕ) to the saccade endpoint (θ), but that neither the initial saccade trajectory nor the initial saccade endpoint was different between the two groups for the prosaccade task (Fig. 2-2B). In contrast, two-way ANOVA for the antisaccade task revealed significant main effects of error ($F(1,52) = 21.25, p<0.0001$) and group ($F(1,51) = 4.91, p=0.031$)(Fig. 2-2B). There was no interaction between error and group, but this comparison approached significance ($p=0.14$), suggesting that the endpoint accuracy (θ) was selectively impaired in the FASD group in the antisaccade task (Fig. 2-2B).

2.4.3 Mapping Endpoints: Prosaccade Task

For the prosaccade task, the endpoint of the first saccade in each trial for each participant was mapped and PCA was performed. Children with FASD were significantly less accurate around the target ($t_{26}=2.9, p=0.005$) (Fig. 2-2C), which further illustrates the increased variability of saccade accuracy to a visual target in children with FASD. To better visualize the variance in endpoints, ellipses were drawn around each participant’s mean endpoint by using the mean (center) and 2 standard deviations in x (transverse diameter) and y (conjugate diameter) directions (Fig. 2-2C inset; contrast red dashed ellipse, FASD, to solid blue ellipse, Control).
2.4.4 Initial Trajectory and Saccade Endpoint: Memory-Guided Task

The outcome measures (Table 2-2) obtained for the memory-guided saccade task were first analyzed using a 2-way repeated measures ANOVA with group (FASD, control) as the between-subject factor and delay (0-1800ms) as the within-subject factor. There was a significant main effect of delay \((F(1,194) = 13.58, p<0.0001)\) only for the percent of timing errors, in that both groups exhibited increased timing errors as the delay was increased (data not shown). However, there was no interaction between group and delay \((p>0.05)\), indicating that the effect of delay was not different between the two experimental groups. The accuracy and trajectory of saccades were also analyzed in this way but there was no main effect of delay. Thus, for simplicity of presentation the data were collapsed across all delays for subsequent analyses. Similar to the prosaccade and antisaccade task, the children with FASD exhibited differences in saccade metrics. Two-way repeated measures ANOVA revealed significant main effects of error \((F(1,51) = 20.42, p<0.0001)\) and group \((F(1,51) = 4.59, p=0.037)\)(Fig. 2-3A). There was no interaction between these factors, suggesting that children with FASD exhibit an overall impairment of accuracy in this task. Additionally, the saccade endpoint to the second target was significantly less accurate in the FASD group compared to the control group \((t_{52}=2.8, p=0.006)\) (Fig. 2-3B).
<table>
<thead>
<tr>
<th>Measure</th>
<th>Prosaccade</th>
<th>Antisaccade</th>
<th>Memory-guided</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>FASD</td>
<td>Control</td>
</tr>
<tr>
<td>Peak Velocity (°/s)</td>
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<td>320 ± 10</td>
<td>357 ± 14</td>
</tr>
<tr>
<td>Amplitude (°)</td>
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<td>8.8 ± 0.2</td>
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</tr>
<tr>
<td>SRT 1st saccade</td>
<td>165 ± 6</td>
<td>164 ± 5</td>
<td>268 ± 8</td>
</tr>
<tr>
<td>SRT 2nd saccade</td>
<td>N.D.</td>
<td>N.D</td>
<td>N.D</td>
</tr>
<tr>
<td>Anticipatory (%)</td>
<td>12 ± 2</td>
<td>15 ± 2</td>
<td>8 ± 2</td>
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<tr>
<td>Corrective (%)</td>
<td>29 ± 3</td>
<td>42 ± 4</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>Direction Errors (%)</td>
<td>0 ± 0.1</td>
<td>0 ± 0.2</td>
<td>34 ± 4</td>
</tr>
</tbody>
</table>
Figure 2-3 Accuracy measures in the memory-guided task. Data are mean ± s.e.m. for participants in the FASD (n=27) and control (n=27) groups. A: Accuracy of the initial trajectory towards the target and accuracy of the initial saccade endpoint towards the target. Solid blue line indicates control group and dashed red line indicates FASD group. B: Accuracy of the final fixation. The effect size \((d)\) is -0.79. *\(p<0.05\) compared with control participants
2.4.5 Cognitive Measures

In agreement with our previous studies (Green et al. 2007; 2009), there was a statistically significant increase in the percent of direction errors during the antisaccade task in the FASD group ($t_{52} = 2.2, p = 0.029$) (Table 2-2). The FASD group corrected these direction errors on average 92% of the time, thereby demonstrating that they understood the task. The memory-guided task revealed significant differences between the groups on two new measures not previously reported in the FASD population. The SRT of the saccade to the second target was significantly faster in children with FASD ($t_{52} = 2.1, p = 0.043$). Since the final fixation, in relation to the second peripheral target, was significantly less accurate in the FASD group this could reflect an inefficient speed-accuracy trade off as the FASD group is faster to the second target but less accurate (Schouten and Bekker, 1967). There was a significant increase in the percentage of timing errors that was found in children with FASD compared to age- and sex-matched control children ($t_{52} = 2.6, p = 0.011$) (Fig. 2-4A). Sequence errors were not different between the two groups (Fig. 2-4B).
Figure 2-4  Executive function measures in the memory guided task. Data are mean ± s.e.m. for subjects in the FASD (n=27) and control (n=27) groups. A: percentage of timing errors in each group. B: percentage of sequence errors in each group. *p<0.05 compared with control subjects.
2.5 Discussion

2.5.1 General Findings

In the current study we performed an in-depth analysis of saccade metrics that revealed new information relating to the quality of saccade motor movements in children with FASD. The results of this study demonstrate that children with FASD exhibit deficits in saccade accuracy in the prosaccade, antisaccade, and memory-guided saccade tasks. Specifically, during the prosaccade task children with FASD were more variable in the endpoints of saccades to a peripheral target, which was reflected by statistically significant increases in the area bounded by the initial saccade endpoints and the frequency of additional, corrective saccades required to achieve the peripheral target. Increasing task complexity increased both the initial and endpoint error in both groups, but children with FASD presented with a greater degree of error in the antisaccade and memory-guided saccade task compared to controls. The memory-guided task required more cognitive processing than the prosaccade task because it was necessary for participants to remember multiple target locations. The antisaccade task required more cognitive demand than the prosaccade task because it requires top-down inhibition and sensory-motor remapping (Munoz and Everling, 2004). The pattern of deficits in eye movement control exhibited by the FASD group is discussed in the context of the brain structures known to underlie control of saccade accuracy and those implicated in FASD.

In agreement with our previous studies (Green et al., 2007; 2009), there were no differences in either amplitude or velocity of saccades in children with FASD compared with controls. However, the deficits in saccade metrics revealed in the current study may have implications for the efficiency with which children with FASD scan visual scenes. We recently reported that children with FASD exhibit differences in both bottom-up and top-down control of
saccades during viewing of natural scenes (Tseng et al., 2013), and that these behavioural differences may be used to differentiate children with FASD from both control children and children with ADHD. One difference from the data reported by Green and colleagues (2009) is that there were no differences observed in SRT for either the prosaccade or antisaccade tasks in the children with FASD. This is most likely a reflection of differences in methodology and/or in the composition of the clinical group. In our previous study, we employed both gap and overlap conditions for the prosaccade and antisaccade tasks, in which the central fixation point either remained illuminated (overlap condition) or disappeared 200ms before the appearance of the peripheral target (gap condition). The gap condition served as an external cue that the peripheral target was about to appear, and in this condition both control children and children with FASD had significantly faster SRTs compared with the overlap condition (Green et al., 2009). Moreover, children with a diagnosis of Alcohol Related Neurodevelopmental Disorder (ARND) (the majority of children in the current study) exhibited minimal changes in SRT relative to control children (Green et al., 2009). Given these two factors it is perhaps not surprising that no differences in SRT were observed between children with FASD and controls.

2.5.2 Eye Movement Circuitry

The superior colliculus, cerebellum and brainstem participate in circuits necessary for saccade execution and online correction of saccade amplitude (Quaia et al., 1999; Robinson et al., 1993; 2002; Takagi et al., 1998). The signal generating a saccade of specific displacement comes from the superior colliculus (Goossens and van Opstal, 2006; Quaia et al., 1999; Sparks et al., 1976) and is fed to the pontine burst generator directly (Buttner-Ennever, 2008; Rodgers et al., 2006) and indirectly through the cerebellar oculomotor vermis (Scudder et al., 2002). The cerebellum is believed to be important for stopping and steering of saccades in order to optimize
accuracy and consistency (Quaia et al., 1999). The cerebellum receives oculomotor input from the pontine nuclei, which project mainly to the posterior lobe of the cerebellar vermis (Scudder et al., 1996). Purkinje cells in the vermis then project to the caudal fastigial nucleus which innervates the excitatory and inhibitory burst neurons and omnipause neurons of the brainstem oculomotor circuitry that control saccades (Robinson and Fuchs, 2001; Scudder et al., 2002). The cerebellum controls saccade accuracy by monitoring motor commands via a feedback model (Quaia et al., 1999; Robinson, 1975) in which internal feedback of a motor command (efference copy) corrects for anticipated errors by rapid modifications of saccade duration (Chen-Harris et al., 2008; Golla et al., 2008; Xu-Wilson et al., 2009). Decreased volume of the cerebellum has been described in studies of individuals diagnosed with FASD (Archibald et al., 2001; Autti-Rämö et al., 2002; Mattson et al., 1992), with some of the changes localized to the anterior vermis (Autti-Rämö et al., 2002; Sowell et al., 1996). It seems likely that damage to any of these structures may underlie the behavioral and accuracy deficits observed in the present study.

2.5.3 Corrective Saccades

The increased percentage of corrective saccades observed in the FASD group could be the result of cerebellar dysfunction. Following dysmetric primary saccades in patients with cerebellar lesions, additional saccades are generated to try to bring the visual target to the fovea (Gaymard et al., 1994). The caudal fastigial nucleus and cerebellar oculomotor vermis are necessary for error correction and rapid adaptation of saccade amplitude (Golla et al., 2008). Injection of muscimol, a GABA<sub>A</sub> receptor agonist, into the caudal fastigial nucleus in monkeys results in perturbations of saccade trajectory (Goffart et al., 2004). Lesions to the caudal fastigial nucleus lead to dysmetria, (Quinet and Goffart, 2004; Straube et al., 2009). Children with FASD were less accurate in looking to the peripheral target in the prosaccade task, but did in fact
correct for the inaccuracy detected in the initial saccades with additional saccades to the goal. The antisaccade and memory-guided tasks did not show this increase in corrective saccades, which may be attributable to the absence of a visual target.

2.5.4 Saccade Endpoint Accuracy

Both the posterior parietal cortex (Wager and Smith, 2003) and the dorsolateral prefrontal cortex (Muri et al., 1996; Pierrot-Deseilligny et al., 1991) have been implicated in the accuracy of memory-guided saccades. Transcranial magnetic stimulation and lesions of the dorsolateral prefrontal cortex impairs the accuracy of memory-guided saccades (Muri et al., 1996; Pierrot-Deseilligny et al., 1991). MR imaging has shown that the parietal lobes are more affected by prenatal alcohol exposure than the temporal and occipital lobes (Archibald et al., 2001). In the memory-guided task, the accuracy of the closest fixation to the first target was not significantly different in the FASD group but the closest fixation to the second target was less accurate in the FASD group compared to the control group. This may indicate a more rapid decline of the memory trace coding the precise location of the second target, perhaps due to a deficit in spatial working memory.

In addition, the FASD participants may have had difficulty updating the location of the second target following the first saccade. The parietal cortex plays a key role in target remapping and the parietal lobes are involved in registering the amplitude and direction of a saccade into the contralateral field, and using that information to update the representation of the location of the next saccade target (Duhamel et al., 1992).

Children in the FASD group exhibited greater deficits in saccade accuracy in the memory-guided saccade task than the simple prosaccade task. The dorsolateral prefrontal cortex is implicated in both short-term spatial memory and response inhibition and in humans with
lesions to the dorsolateral prefrontal cortex, increased variability is observed in the accuracy of memory-guided saccades, suggesting its role in the encoding of spatial information (Pierrot-Deseilligny et al., 2003). Additionally, as children with FASD tend to exhibit greater deficits in tasks of increasing complexity, these results may reflect an inability to store both sequence and location.

The increase in variability in the prosaccade task and the increased deficit in saccade accuracy for the antisaccade and memory-guided saccade tasks may be due to deficits in connections between higher order brain structures and the cerebellum (Glickstein and Doron, 2008). For example, the cerebellum has indirect connections with cerebral cortex, basal ganglia and thalamus. These pathways could participate in a more enduring resiliency of saccade trajectories due to remapping of the target (Chen-Harris et al., 2008; Soetedjo et al., 2009). A deficit in these areas seems likely as volumetric analysis of MR images showed decreased volume in the diencephalon (thalamus and hypothalamus) in children with FASD (Mattson et al., 1996).

2.5.5 Cognitive Measures

In this study, children with FASD had significantly increased direction errors when compared to age- and sex-matched controls. These deficits have previously been attributed to poor voluntary control over saccade generation in several clinical populations with frontostriatal circuitry impairments, including FASD (Green et al., 2007; 2009). Similar to the antisaccade task, the memory-guided task assesses the executive control of internally-guided saccades. The difference in this task is that participants must use previously presented sensory information to plan and initiate a multi-component motor response, but also wait to receive the appropriate “go” signal before initiating the response. This task therefore requires the integration of multiple
domains of cognitive function, including spatial working memory and response inhibition. In the present study, children with FASD demonstrated increased timing errors in the memory-guided task, reflecting deficits in either the ability to suppress and/or to inhibit saccadic responses. Sequence errors were not different between the groups, suggesting that, for children with FASD, remembering the sequence of two targets is easier to accomplish than waiting for the proper go signal.
2.6 Conclusions

Together, findings from the prosaccade, antisaccade, and memory-guided tasks reveal that performance of these eye movement paradigms may be sensitive to cerebellar dysfunction in children with FASD. Additionally, this dysfunction is amplified when higher cortical structures, also compromised in FASD, are recruited for more complex tasks. This inaccuracy can negatively impact the everyday lives of children with FASD. Activities such as sports, reading, typing, driving, and food preparation become increasingly difficult when the initial saccade is not accurate, and additional corrective saccades must be made (Land, 2006). Therefore, it is important to better characterize these deficits to increase our understandings of the impact they have on children with FASD.
2.7 Acknowledgements

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Chapter 3

Eye Movements Reveal Sexually Dimorphic Deficits in Children with Fetal Alcohol Spectrum Disorder

3.1 Abstract

Background: We examined the accuracy and characteristics of saccadic eye movements in children with fetal alcohol spectrum disorder (FASD) compared with typically developing control children. Previous studies have found that children with FASD produce saccades that are quantifiably different from controls. Additionally, animal studies have found sex-based differences for behavioral effects after prenatal alcohol exposure. Therefore, we hypothesized that eye movement measures in children with FASD and typically developing control children will show sexually dimorphic results.

Methods: Children (aged 5-18 years) with FASD (n=71) and typically developing controls (n=113) performed a visually-guided saccade task. Saccade metrics and behavior were analyzed for sex and group differences.

Results: Female control participants had greater amplitude saccades than control males or females with FASD. Accuracy was significantly poorer in the FASD group, especially in males, which introduced significantly greater variability in the data. Therefore, we conducted additional analyses including only those trials in which the first saccade successfully reached the target within a ±1˚ window. In this restricted amplitude dataset, the females with FASD made saccades with significantly lower velocity and longer duration, whereas the males with FASD did not differ from the control group. Additionally, the mean and peak decelerations were selectively decreased in the females with FASD.
Conclusions: These data support the hypothesis that children with FASD exhibit specific deficits in eye movement control and sensory-motor integration associated with cerebellar and/or brain stem circuits. Moreover, prenatal alcohol exposure may have a sexually dimorphic impact on eye movement metrics, with males and females exhibiting differential patterns of deficit.
3.2 Introduction

Prenatal alcohol exposure can cause damage to the developing brain of the fetus and may lead to a range of cognitive deficits that include problems with executive functions, attention, and working memory (Kodituwakku 2009; Mattson et al. 1999; Rasmussen 2005). This can lead to negative behavioral, neuropsychiatric, and maladaptive outcomes commonly observed in this population, which has recently gained greater attention, as neurodevelopmental disorder associated with prenatal alcohol exposure was added to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association 2013; Rasmussen, Andrew, Zwaigenbaum, & Tough 2008; Streissguth, Bookstein, Barr, Sampson, O'Malley, & Young 2004). Fetal alcohol spectrum disorder (FASD) is an umbrella term used to describe the full range of adverse effects induced by prenatal alcohol exposure. FASD has several subtypes that include fetal alcohol syndrome (FAS) and partial fetal alcohol syndrome (pFAS), which presents with full/partial facial dysmorphology, growth deficiency, and central nervous system dysfunction; and alcohol related neurodevelopmental disorder (ARND) which presents with central nervous system dysfunction (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). Many secondary disabilities have been identified in those diagnosed with FASD including mental health disorders, addictions, trouble with the law, and problems with employment (Pei et al. 2011a). Two key protective factors that have been identified as decreasing secondary disabilities include receiving services for developmental disabilities and having a diagnosis before the age of 6 years (Streissguth, Bookstein, Barr, Sampson, O'Malley, & Young 2004). Effective screening tools that can accurately assess brain function in a non-invasive manner could facilitate the early and accurate identification of alcohol-exposed children. Also, by identifying the underlying brain pathology better interventions and services can be developed.
Here, we assess eye movement measures obtained during the performance of a visually-guided saccade task as a possible screening tool for use in children with FASD.

Studies conducted in animal models have found behavioral and physiological sex differences in offspring as a consequence of prenatal alcohol exposure. These studies have found differences in male and female physiological responses to stressors, serotonin, hypothalamic-pituitary-adrenal (HPA) axis function, and behavioral responses in recognition memory and spatial working memory in animals prenatally exposed to alcohol (Goodlett and Peterson 1995; Kelly et al. 2009; Sliwowska et al. 2014; Weinberg et al. 2008; Weinberg and Jerrells 1991). For example, female rats prenatally exposed to alcohol show deficits in their ability to use or respond to environmental cues (Weinberg 1988; Weinberg 1992b), which could translate into deficits in visual response processing. Additionally, several studies have found differences in exploratory eye movements between control males and females when viewing natural images (Mercer Moss et al. 2012; Nishiura et al. 2007). Based on these findings, we sought to investigate if any behavioral eye movement measures displayed an interaction between males and females in control and FASD participants. Due to the above differences found in animal studies and the high precision eye movement control measures have when assessing brain damage, we predicted that the eye movement measures of healthy controls and those with FASD will show sexually dimorphic results which can be used to better characterize children prenatally exposed to alcohol.

Saccades are rapid eye movements that bring visual targets onto the fovea of the retina. This tool was chosen to assess children with FASD because the measurement of eye movements can be used to differentiate disorders of the nervous system by assessing sensory, motor, and cognitive function (Munoz et al. 2007; Ramat, Leigh, Zee, & Optican 2007). The eye movement system has also been used to characterize healthy development throughout childhood (Alahyane
et al. 2014; Luna et al. 2001; Luna et al. 2008; Munoz et al. 1998). Important features of saccadic eye movements are the metrics, which describe the accuracy and quality of the motor processes. One of the most commonly studied features of saccade metrics is the main sequence which examines the relationship between amplitude, velocity and duration (Bahill et al. 1975; Leigh & Zee 2006). Examining metric measures and main sequence relationships may reveal important information about brain function, as the brain regions involved are well characterized (Garbutt et al. 2001; Leigh & Zee 2006; Scudder et al. 2002; Sparks 2002).

Eye movement control is a reliable and accurate measure of prenatal alcohol exposure and can differentiate those with FASD from typically developing controls (Green, Munoz, Nikkel, & Reynolds 2007; Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009a; Paolozza et al. 2013c; Paolozza et al. 2014a; Paolozza et al. 2014b; Tseng, Cameron, Pari, Reynolds, Munoz, & Itti 2013a; Tseng et al. 2013c). One task that has revealed differences between FASD and control participants is the prosaccade task which requires participants to make visually-guided saccades to peripheral targets (Johnson et al. 2012; Munoz and Everling 2004). In our previous studies, children with FASD were shown to have significantly poorer accuracy in the prosaccade task, with more variable saccade endpoints, and increases in saccade endpoint deviation and the frequency of additional, corrective saccades required to achieve final fixation (Paolozza, Titman, Brien, Munoz, & Reynolds 2013c). Moreover, this increased saccade endpoint deviation correlated with poorer visuospatial processing on a psychometric task of line orientation judgment (Paolozza, Rasmussen, Pei, Hanlon-Dearman, Nikkel, Andrew, McFarlane, Samdup, & Reynolds 2014b). Therefore, reduced ability to control saccade accuracy is an important feature of the FASD behavioral phenotype. Here, we investigate the metrics of visually-guided saccades in a large group of both children.
with FASD and healthy controls to test the hypothesis that children with FASD will exhibit
dysfunction in the cerebellum and/or brainstem components of the saccade control circuit, as
evidenced by deviations from normal saccade metrics. This study had two objectives: to examine
group differences in saccade metrics in children with FASD compared to healthy controls and to
test for sex differences in these two groups.

3.3 Methods

3.3.1 Participants

Participants aged 5-18 years were recruited from five sites across Canada. Children with
FASD (n=71; mean age 11.8±0.4) were previously assessed and diagnosed according to the
Canadian Guidelines for FASD Diagnosis (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc
2005) and were recruited through diagnostic clinics in Kingston, ON; Ottawa, ON; Edmonton,
AB; Cold Lake, AB; and Winnipeg, MB, as part of a larger Canada-wide network study funded
by NeuroDevNet (Reynolds et al. 2011). Typically developing children (n=113; mean age
10.3±0.3) were recruited from the same geographical areas and were excluded if they had any
neurological or psychiatric disorder, or visual disturbance, other than requiring corrective lenses.

All experimental procedures were reviewed and approved by the Human Research Ethics Boards
at Queen’s University (Kingston), University of Alberta (Edmonton and Cold Lake), Children’s
Hospital of Eastern Ontario (Ottawa), and the University of Manitoba (Winnipeg). Written
informed consent was obtained from a parent or legal guardian and assent was obtained from
each child before study participation. Demographic information is summarized in Table 1.
Socioeconomic status (SES) was calculated using Hollingshead’s Four-Factor Index of Social
Status (Hollingshead 2011). Study data were collected and managed using REDCap electronic
data capture tools (Harris et al. 2009).
Table 3-1 Demographic variables for control and FASD groups

<table>
<thead>
<tr>
<th></th>
<th>Control (n=113)</th>
<th>FASD (n=71)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males (%)</td>
<td>53 (47)</td>
<td>39 (55)</td>
<td>0.65</td>
</tr>
<tr>
<td>Females (%)</td>
<td>60 (53)</td>
<td>32 (45)</td>
<td>0.38</td>
</tr>
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<tr>
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<td></td>
</tr>
<tr>
<td>Partial Fetal Alcohol Syndrome (%)</td>
<td>-</td>
<td>14 (20)</td>
<td></td>
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<tr>
<td>Alcohol Related Neurodevelopmental Disorder (%)</td>
<td>-</td>
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<tr>
<td>Comorbidities:</td>
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<td></td>
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<tr>
<td>Attention Deficit Hyperactivity Disorder (%)</td>
<td>-</td>
<td>43 (61)</td>
<td></td>
</tr>
<tr>
<td>Anxiety (%)</td>
<td>-</td>
<td>9 (13)</td>
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</tr>
<tr>
<td>Oppositional Defiant Disorder (%)</td>
<td>-</td>
<td>7 (10)</td>
<td></td>
</tr>
<tr>
<td>Depression (%)</td>
<td>-</td>
<td>6 (8)</td>
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</tr>
<tr>
<td>Other (%)</td>
<td>-</td>
<td>19 (26)</td>
<td></td>
</tr>
<tr>
<td>Medications:</td>
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<tr>
<td>Stimulants (%)</td>
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<td>31 (43)</td>
<td></td>
</tr>
<tr>
<td>Antipsychotics (%)</td>
<td>-</td>
<td>17 (24)</td>
<td></td>
</tr>
<tr>
<td>Antidepressants (%)</td>
<td>-</td>
<td>8 (11)</td>
<td></td>
</tr>
<tr>
<td>Other (%)</td>
<td>-</td>
<td>14 (19)</td>
<td></td>
</tr>
<tr>
<td>Age (years±SD)</td>
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<td>Socioeconomic Status:</td>
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<td>41±14</td>
<td>0.0096</td>
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<tr>
<td>First Nations/Metis (%)</td>
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<td>43 (61)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Caucasian (%)</td>
<td>106 (94)</td>
<td>25 (35)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Other (%)</td>
<td>5 (4)</td>
<td>3 (4)</td>
<td>ns</td>
</tr>
</tbody>
</table>

The control group had no comorbidities or medications except asthma and requiring an inhaler (n=3). ns=not significant
3.3.2 Saccadic eye movement recordings

Participants were seated comfortably in a dark, quiet room on a stationary chair and instructions for each trial were given verbally, and repeated back to the experimenter by the participant. Eye position was recorded using the Eyelink 1000 (SR Research, Kanata, Canada). A 17” LCD monitor and mounted infrared camera were at a distance of 58-64cm from the left eye. The position of the left pupil was digitized in both the vertical and horizontal axes at a sampling rate of 500 Hz. Saccades were defined as having a speed of greater than 2.5 times the standard deviation of the background noise (measured during fixation) for at least five sample points. Before each task the eye movements of each participant were calibrated using nine screen targets (eight around the periphery and one central) of known position. This ensured that the participants had no visual disturbances that would impair task performance as they would be unable to orient their eyes to the target positions correctly.

Each trial started with illumination of a central fixation point (FP) for 800-1200ms. The FP then disappeared and, after a 200ms delay (gap period), a peripheral target appeared randomly at 10° to the left or right of the FP. Participants were given 1000ms to initiate and complete a saccade towards the target. No feedback was given about performance. The gap period was employed because it produces the shortest SRT (Dorris and Munoz 1995; Fischer & Ramsperger 1984; Saslow 1967). One block of 60 trials was obtained from each participant as part of a larger battery of eye movement and psychometric tests. The entire testing session was two hours and participants were compensated with gift cards. Testing was kept to a maximum of two hours to minimize fatigue. We have previously reported on other measures of this battery in this cohort (Paolozza, Titman, Brien, Munoz, & Reynolds 2013c; Paolozza, Rasmussen, Pei,
3.3.3 Data Analysis

Data were analyzed using custom software developed in MATLAB (Mathworks, Natick, Massachusetts). Only correct trials in which the participants fixated on the FP at the start of the trial and made a saccade in response to the target appearance were included in the analysis. On average 91% of trials were viable (53-100% range).

For correct trials, saccadic reaction time (SRT) was defined as the time from the appearance of the peripheral target to the initiation of the first saccade. The deviation of the saccade endpoint was defined as the angular distance between the ideal path from fixation to target and the trajectory of the first saccade toward the goal by drawing a straight line from the beginning to the end of the saccade (Paolozza, Titman, Brien, Munoz, & Reynolds 2013c). Amplitude, peak velocity, duration, peak acceleration, and peak deceleration were calculated for each correct trial. The mean acceleration (the slope of the velocity plot from saccade onset to peak velocity) and mean deceleration (the slope from peak velocity to saccade termination) were also calculated. A skew index was calculated from mean acceleration (slope 1) and deceleration (slope 2) using the following equation: skew index=(slope 1-slope 2)/(slope 1+slope 2). A positive skew indicated that mean acceleration (slope 1) was steeper than mean deceleration (slope 2), whereas a negative skew indicated that mean deceleration (slope 2) was steeper than mean acceleration (slope 1). A skew index of 0 indicated that both slopes were the same.

A separate analysis was performed to explore potential covariates associated with demographic factors (Table 1) by first examining the data for trends/disparities and then running the appropriate statistical tests. All data were also examined for outliers and if a data point was
found to be greater than two standard deviations away from the mean of that group they were excluded.

The control group data were first analyzed by performing Pearson correlations between each saccade measure and age. If the measure varied significantly with age, then age corrections were performed. Due to the large number of control participants it was possible to perform age-correction by calculating a standardized t-score equation for each individual age. Age-corrected scores for the FASD group were then calculated using the t-score equation obtained from the control group. Interactions between sex and group were analyzed using two-way, two-tailed repeated measures analysis of variance (ANOVA). Tukey’s post-hoc test for multiple comparisons with adjusted p-values was used for those outcomes in which a group difference and interaction were found to compare all groups with each other. Sidak’s post-hoc test for multiple comparisons with adjusted p-values was used for only those outcomes in which an interaction was found to compare the control and FASD group. Pearson correlations between the main sequence measures were also performed using individual trial data to allow for a range of amplitudes to be measured. The correlation coefficients were then compared between the FASD and control group by using the Fisher r-to-z transformation.

In the current dataset, the accuracy of the saccade endpoint and amplitude were significantly poorer in the FASD group, which introduced significantly greater variability and statistically greater variances. Therefore, we conducted additional analyses including only those saccades in which amplitude and endpoint fell within 9±1° on the horizontal axis and ±1° on the vertical axis. These values were selected because they reflected the mean values of control participants, fitting with the tendency for initial saccades to be slightly hypometric (Leigh & Zee
2006). Interactions between sex and group were analyzed as described above for the unrestricted data using two-way ANOVA.
3.4 Results

3.4.1 Demographic Findings

Differences in sex distribution between the two groups were calculated by performing Fisher’s exact test for both groups and no significant differences were found. FASD subtype was investigated by dividing the FASD group into two subgroups (FAS/pFAS and ARND) and comparing these subgroups to the control group on all outcome measures using a one-way ANOVA. No significant differences were detected indicating the ARND group performed similarly to the FAS/pFAS group. Due to the relatively low number of most comorbidities in the FASD group, only attention deficit hyperactivity disorder (ADHD) could be properly investigated. This was accomplished by dividing the FASD group into those with ADHD and those without and comparing the two groups on the metric measures using a t-test. No significant differences were found indicating that a comorbidity of ADHD did not affect the data for this cohort. Age was analyzed via a t-test and the control group was found to be significantly younger than the FASD group \((t(179)=3.37, p=0.0009)\). However, we controlled for age by performing age corrections on those outcome measures that changed significantly with age. Next, SES was investigated using a t-test to compare the two groups. The FASD group was found to have significantly lower SES compared to controls \((t(125)=2.63, p=0.0096)\). However, when correlations were run between SES score and each eye movement score no significant relationships were detected indicating that SES did not affect metric scores in this cohort. Finally, ethnicity was examined in the same way as ADHD by dividing the FASD group into those identified as First Nations and those with any other ethnicity (primarily Caucasian). Using a t-test, no significant differences were found between the two groups. Therefore, diagnostic
subgroup, comorbidities, SES, and ethnicity did not influence the data for this cohort and did not need to be included as covariates.

3.4.2 Overall Metric Findings

In both typically-developing and FASD participants, the best-fit lines of the main sequence relationships were linear for each participant (Fig. 1A provides examples). Pearson’s correlation revealed significant positive relationships in both groups, with amplitude-velocity (control: $r=0.644$, $p<0.0001$; FASD: $r=0.613$, $p<0.0001$) and amplitude-duration (control: $r=0.471$, $p<0.0001$; FASD: $r=0.538$, $p<0.0001$) exhibiting the strongest relationships, followed by duration-velocity (control: $r=0.136$, $p<0.0001$; FASD: $r=0.103$, $p<0.0001$). The slopes of the main sequence relationships were calculated separately for each participant. The mean slope of the amplitude-velocity relationship was different between groups with the FASD group displaying a significantly lower slope ($t(180)=2.413$, $p=0.0168$; Fig. 1B). Thus, saccades produced by children with FASD tended to be slower than saccades produced by controls. The mean slope of the amplitude-duration relationship was not significantly different (data not shown).
Figure 3-1 Main sequence relationships. (A) The velocity-amplitude relationship of a 15-year-old control participant and 15-year-old FASD participant. (B) Data for participants in the FASD group (n=71) shown in red and the control group (n=113) shown in blue. The slope of the velocity-amplitude relationship was significantly lower in the FASD group. Control is shown in blue and FASD is shown in red. *, p<0.05
Several behavioural sexually dimorphic effects were found both within and between groups (Table 2). There was a significant interaction between group and sex (F(1,182)=4.12, p=0.044) for SRT (Fig. 2A), and the post-hoc test revealed that males with FASD were slower than control males (p=0.043). A main effect of group (F(1,180)=4.016, p=0.047) and an interaction between group and sex (F(1,180)=12.54, p=0.0005) were found for amplitude (Fig. 2B). Post-hoc analysis revealed that females in the control group had greater saccade amplitude compared to control males (p=0.020) and females with FASD (p=0.0009). Main effects of group (F(1, 182)=12.11, p=0.0006) and sex (F(1,182)=6.39, p=0.012), and an interaction (F(1, 182)=4.97, p=0.027) were found for saccade endpoint angle of error (Fig. 2C). The post-hoc test revealed that males with FASD had greater endpoint angle of error compared to control males (p=0.0037) and both control females (p>0.0001) and females with FASD (p=0.0023).
Table 3-2 Sex Differences between FASD and controls

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control (mean±SEM)</th>
<th>FASD (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>SRT t-score</td>
<td>48.7±1</td>
<td>51.5±1</td>
</tr>
<tr>
<td>Amplitude t-score</td>
<td>47.1±1*</td>
<td>53.1±1</td>
</tr>
<tr>
<td>Endpoint t-score</td>
<td>51.3±1</td>
<td>48.8±1</td>
</tr>
</tbody>
</table>

*indicates significant difference from control females, †indicates significant difference from control males
Figure 3-2 Overall metric data. Data are mean ± SEM for participants in the FASD group (n=71) shown in red and the control group (n=113) shown in blue. (A) Males with FASD had significantly slower saccadic reaction time (SRT) compared to control males. (B) Control females had significantly greater amplitude compared to control males and females with FASD. (C) Males with FASD had significantly greater endpoint angle of error compared to all other groups. *, p<0.05 compared to group indicated or all other groups.
3.4.3 Amplitude Restricted Data Findings

Due to the group difference we observed in amplitude and endpoint error, a proper metrics analysis could not be conducted. However, by matching saccade amplitude, the velocity and duration could be further examined. We restricted the amplitude range to $9 \pm 1^\circ$ and found that several group and sex differences emerged between the control and FASD groups (Table 3). There were no significant differences found for SRT. As expected, there was a significant interaction ($F(1,180)=5.19, p=0.024$) found for amplitude, but no post-hoc group or sex differences. There was a main effect of group ($F(1,180)=5.67, p=0.018$) and an interaction between group and sex ($F(1,180)=5.05, p=0.026$) found for peak velocity (Fig. 3). The post-hoc test revealed that the females in the FASD group had lower peak velocity when compared to control females ($p=0.0087$). There was also a main effect of group ($F(1,180)=4.20, p=0.042$) found for duration with the FASD group displaying longer duration; however, there was no effect of sex or interaction between groups. Peak acceleration was not different between the groups, but a significant interaction between group and sex ($F(1,180)=4.55, p=0.0344$) was found for peak deceleration (Fig. 3). The post-hoc test revealed that females in the FASD group had slower peak deceleration compared to control females ($p=0.030$). There were no significant results found for mean acceleration, but there was a significant interaction between group and sex for mean deceleration ($F(1,180)=5.95, p=0.0157$). The post-hoc test again revealed that FASD females had a decreased mean deceleration compared to control females ($p=0.0072$). There was a main effect of group ($F(1,180)=5.86, p=0.023$) and an interaction ($F(1,180)=4.01, p=0.047$) for skew index. The females with FASD had a greater skew index compared to both control males ($p=0.020$) and females ($p=0.037$). There were no significant results found for saccade endpoint error. Therefore, once amplitude was matched between the two groups several
metric deficits were found between groups with the females with FASD displaying increased vulnerability.
Table 3-3 Sex differences between FASD and control for amplitude restricted dataset

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control (mean±SEM)</th>
<th>FASD (mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Velocity (°/s)</td>
<td>327±4.9</td>
<td>339±4.9</td>
</tr>
<tr>
<td>Amplitude (°)</td>
<td>9.0±0.04</td>
<td>9.2±0.04</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>49.7±0.8</td>
<td>48.8±0.5</td>
</tr>
<tr>
<td>Amplitude (°)</td>
<td>9.0±0.04</td>
<td>9.2±0.04</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>49.7±0.8</td>
<td>48.8±0.5</td>
</tr>
<tr>
<td>Acceleration (°/s²)</td>
<td>23958±551</td>
<td>25527±555</td>
</tr>
<tr>
<td>Deceleration (°/s²)</td>
<td>-19706±428</td>
<td>-20823±430</td>
</tr>
<tr>
<td>Slope 1</td>
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<td>14.2±0.3</td>
</tr>
<tr>
<td>Slope 2</td>
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<td>11.1±0.3</td>
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<tr>
<td>Skew Index</td>
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<td>0.12±0.01</td>
</tr>
<tr>
<td>SRT t-score</td>
<td>163.5±5.4</td>
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</tr>
<tr>
<td>Endpoint (°)</td>
<td>2.5±0.1</td>
<td>2.5±0.1</td>
</tr>
</tbody>
</table>

*indicates significant difference from control females
Figure 3-3 Schematic of metric measures. Individual data for each participant are shown in pink for the participants with FASD (n=71) and light blue for the controls (n=113). The mean of the FASD group is shown as a bold red line and control group as a bold blue line. Males (control vs. FASD) are on the left and females (control vs. FASD) are on the right. (E) Eye traces of correct saccades when participants look from a central fixation point to a peripheral target. (È) Velocity profiles of the control and FASD participants. Peak velocity was significantly slower in the females with FASD compared to control females. (Ê) Acceleration and deceleration profiles of all participants. Peak deceleration, but not acceleration, was significantly slower in the females with FASD compared to control females. *Significant results (p<0.05) are indicated by black dotted circle.
3.5 Discussion

3.5.1 Overall Findings

The first objective of this study was to perform a more extensive examination of saccade metrics, in particular the main sequence, in children with FASD compared with typically developing controls. We found that children with FASD exhibited an alteration in the main sequence, specifically decreased slope of the velocity-amplitude relationship. This indicates that these two measures are linearly related in both groups with the FASD group displaying decreased velocity, leading to a decreased slope of the velocity-amplitude relationship. This pattern of deficits is a potential biomarker of impairment in either the brainstem circuitry or cerebellum of FASD participants.

The second objective was to test for sex differences where we found that males with FASD had slower SRT and greater saccade endpoint deviation, while the females with FASD had significantly decreased amplitude. When the performance of all amplitude restricted trials was examined, males with FASD did not show any differences from controls, but the females with FASD exhibited lower peak velocity, peak deceleration, and average deceleration. The females with FASD also had increased skewness of the velocity profile. Thus, the potential impairment in cerebellar and/or brainstem circuits may also have a sex component that should not be overlooked.

3.5.2 Behavioural Findings

Amplitude and velocity of saccadic eye movements exhibit a consistent relationship in which larger amplitude eye movements are accompanied by greater peak velocity in healthy controls (Bahill, Clark, & Stark 1975; Boghen et al. 1974; Leigh & Zee 2006). This same main sequence relationship was found for both the FASD and control groups, but the slope of this
relationship was reduced for the FASD group. Saccade duration is also linearly related to amplitude (Bahill, Clark, & Stark 1975; Baloh et al. 1975; Leigh & Zee 2006). Again, this relationship held for both the FASD and control groups but no difference in slope was found between the two groups. This is not surprising because saccade duration was not significantly different between the two groups and a large range of amplitudes was not examined.

Only a few recent studies have reported sexually dimorphic differences in humans with FASD (e.g., Dodge et al. 2014; Fuglestad et al. 2014), however none have examined eye movement behaviours. Additionally, many animal studies have found both physiological and behavioral sex-dependent differences when examining prenatal alcohol exposure. For example, female rats prenatally exposed to alcohol have been found to have enhanced response to stressors (Halasz et al. 1993) and show deficits in their ability to use or respond to environmental cues. Increased impairment in female animals was also found for spatial working memory after prenatal alcohol exposure (Weinberg 1992a; Weinberg 1992b). Finally, differences in recognition memory and social cues have also been found to be increased in female, but not male, rats exposed prenatally to alcohol (Kelly, Leggett, & Cronise 2009). These animal findings and the small number of human studies examining sex differences led us to investigate if any eye movement measures distinguished males and females prenatally exposed to alcohol.

Interestingly, both males and females with FASD did show sexually dimorphic results on prosaccade eye movement measures. The mechanism of how this occurs is unknown but animal studies have suggested that prenatal alcohol exposure may be altering the gonadal-adrenal interactions during fetal development (Carter et al. 2014; Weinberg, Sliwowska, Lan, & Hellemans 2008). In our study, males with FASD had slower reaction times compared to control males. Slower saccadic reaction times have been previously found in a different population of
children with FASD; however, interactions between sexes were not investigated (Green, Munoz, Nikkel, & Reynolds 2007; Green et al. 2009). Additionally, slower reaction time have also been found in children with FASD when completing other tasks (e.g. Burden et al. 2005; Jacobson et al. 1994; Kable and Coles 2004). Increases in SRT can be caused by damage to many different structures in the brain and can also indicate diffuse cortical damage in regions such as the occipital, frontal, and parietal lobes (Leigh & Zee 2006). Therefore, it appears that males with FASD may have less specific but more widespread and diffuse damage due to prenatal alcohol exposure, leading to increased SRT.

Sexually dimorphic results also emerged when saccadic trajectory and accuracy were examined in the unrestricted data. Saccades have very short durations, and because of this there is insufficient time for visual feedback to correct ongoing movements, and therefore inaccuracies can be caused by deficits in internal monitoring (Leigh & Kennard 2004). Increased variability in trajectory can also lead to saccade inaccuracies (Smit & Van Gisbergen 1990; Van der Stigchel, Meeter, & Theeuwes 2006). Finally, saccade inaccuracies can be caused by overshooting (hypermetric) or undershooting (hypometric) the target or object of interest. In this study, females with FASD produced smaller initial amplitude saccades compared to control females, and males with FASD displayed greater endpoint inaccuracy compared to both control males and females. This indicates that whereas both sexes in the FASD group were less accurate, the underlying cause of the inaccuracy differed, suggesting sex differences in brain injury due to prenatal alcohol exposure.

After restricting the data by matching amplitudes, the velocity, duration and saccade waveform could be properly examined (Fig. 3). The analysis revealed a group but not sex difference in duration. There was both a group and sex difference in peak velocity and peak
deceleration with females with FASD displaying a decrease in both. In healthy controls, the skewness of the velocity waveform has been consistently found to be asymmetrical during a horizontal saccade, with a skew to the left (Balogh, Sills, Kumley, & Honrubia 1975; Van Opstal and Van Gisbergen 1987). In the current study, a more positive skew was found for the females with FASD compared to both control males and females. This is caused by the decreased mean deceleration of the velocity profile.

3.5.3 Neural Mechanisms Associated with Saccade Impairments

The saccade inaccuracies observed in the initial saccades of the unrestricted dataset implicate the cerebellum (Collins et al. 2008; Crawford & Guitton 1997; Keller, Slakey, & Crandall 1983; Leigh & Zee 2006). The nucleus reticularis tegmenti pontis encodes the size and direction of saccades in three dimensional eye displacement vectors (Van, Hepp, Suzuki, & Henn 1996). It projects to the dorsal vermis and caudal fastigial nucleus of the cerebellum. The dorsal vermis is involved in modulating on-line amplitude and trajectory during a saccade (Keller, Slakey, & Crandall 1983). The fastigial nucleus works with the dorsal vermis to control saccade accuracy by monitoring motor commands via internal feedback of the desired and ongoing motor command (efference copy) and corrections for anticipated errors are produced by subtle yet rapid modifications of saccade duration (Robinson & Fuchs 2001). These connected structures play a critical role in saccade metrics and accuracy and we propose that they appear to be impacted by prenatal alcohol exposure.

After restricting the dataset and matching saccade amplitude, the neural correlates of both velocity and duration could be examined in greater detail. The decreased peak velocity observed in females with FASD may indicate damage to the brainstem itself or its connections because the pons is critical for the generation of saccades. Specifically, excitatory burst neurons in the
paramedian pontine reticular formation are essential for driving the initial acceleration to generate a horizontal saccade (Leigh & Zee 2006; Scudder, Moschovakis, Karabelas, & Highstein 1996). Therefore, the decreased peak velocity found in females with prenatal alcohol exposure may be due to damage to the paramedian pontine reticular formation.

In addition to the decreased peak velocity observed in the females with FASD, it also appears that when the saccade waveform was analyzed in the restricted dataset, females with FASD had deficits specific to the latter half of the saccade. Inactivation of the caudal fastigial nucleus using muscimol is known to decrease deceleration with little to no effect on acceleration (Goffart et al. 2004; Robinson et al. 1993). The caudal fastigial nucleus projects to the burst neurons in the pons and this projection may be impaired in females with FASD (Buzunov et al. 2013; Goffart, Chen, & Sparks 2004). The deficit in the peak and mean deceleration in the females with FASD is indicative of cerebellar damage in the caudal fastigial nucleus or its projections to the brainstem.
3.6 Conclusions

The findings reported here indicate that children with FASD have deficits in multiple measures of saccade performance including accuracy, main sequence, and SRT. Additionally, we conclude, for the first time, that prenatal alcohol exposure has a sexually dimorphic impact on eye movement control with females exhibiting greater metric vulnerability and males exhibiting greater variability in saccadic reaction times and online error correction. These findings implicate impairment in cerebellar and/or brain stem circuits. The next steps will be to combine the current measures with MRI studies to better characterize the neural correlates of the sexually dimorphic results. Additionally, males and females with FASD appear to have distinct patterns of saccade measure deficits that may be used to screen for those prenatally exposed to alcohol using a simple 5 minute saccade task. Earlier diagnosis has been found to be crucial for children with FASD because it leads to better outcomes later in life due to the recognition and treatment for neurological, behavioural, and mental health issues faced by these children.
3.7 Acknowledgments:

We thank the participants and their families for taking part in the study. This work was supported by NeuroDevNet, which is funded by the Networks of Centres of Excellence, a program of the federal government to advance science and technology. We also acknowledge the NeuroDevNet NeuroInformatics Core for data management system implementation and support and Donald Brien for his technical expertise in the collection and analysis of the eye movement data. DPM is supported by the Canada Research Chair Program.
Chapter 4

Deficits in response inhibition correlate with oculomotor control in children with Fetal Alcohol Spectrum Disorder and prenatal alcohol exposure

4.1 Abstract

Children with Fetal Alcohol Spectrum Disorder (FASD) or Prenatal Alcohol Exposure (PAE) frequently exhibit impairment on tasks measuring inhibition. The objective of this study was to determine if a performance-based relationship exists between psychometric tests and eye movement tasks in children with FASD. Participants for this dataset were aged 5-17 years and included those diagnosed with an FASD (n=72), those with PAE but no clinical FASD diagnosis (n=21), and typically developing controls (n=139). Participants completed a neurobehavioral test battery, which included the NEPSY-II subtests of Auditory Attention, Response Set, and Inhibition. Each participant completed a series of saccadic eye movement tasks, which included the antisaccade and memory-guided tasks. Both the FASD and the PAE groups performed worse than controls on the subtest measures of attention and inhibition. Compared with controls, the FASD group made more errors on the antisaccade and memory-guided tasks. Among the combined FASD/PAE group, inhibition and switching errors were negatively correlated with direction errors on the antisaccade task but not on the memory-guided task. There were no significant correlations in the control group. These data suggest that response inhibition deficits in children with FASD/PAE are associated with difficulty controlling saccadic eye movements which may point to overlapping brain regions damaged by prenatal alcohol exposure. The results of this study demonstrate that eye movement control tasks directly relate to outcome measures.
obtained with psychometric tests that are used during FASD diagnosis, and may therefore help with early identification of children who would benefit from a multidisciplinary diagnostic assessment.
4.2 Introduction

Prenatal exposure to alcohol is the leading, preventable cause of neurobehavioural dysfunction in children. The full spectrum of adverse effects induced by prenatal alcohol exposure, which includes several diagnostic subgroups, is collectively referred to as Fetal Alcohol Spectrum Disorder (FASD) (Chudley et al. 2005). FASD is estimated to occur in 1% of the population in North America (May, Gossage, Kalberg, Robinson, Buckley, Manning, & Hoyme 2009). Children with FASD often present with a wide variety of mild to severe deficits including problems with executive functions (e.g., response inhibition, planning, and cognitive flexibility), attention, and working memory (Kodituwakku 2009; Mattson, Crocker, & Nguyen 2011; Rasmussen 2005), which may contribute to the negative behavioural, neuropsychiatric, and maladaptive outcomes commonly observed in this population (Rasmussen, Andrew, Zwaigenbaum, & Tough 2008). This study focused on inhibition deficits, a prevalent problem for those affected by FASD. Poor inhibitory control in children with FASD is likely associated with disruptive behaviors in school and at home (Nigg 2003), and may lead to impulsive behaviors later in life resulting in legal problems, inappropriate sexual behaviour, and substance abuse (Streissguth, Bookstein, Barr, Sampson, O'Malley, & Young 2004).

Inhibition is the ability to suppress irrelevant stimuli or behavioural impulses to enable goal-directed behaviour. Saccades are rapid eye movements that bring new visual targets onto the fovea of the retina. Measurement of eye movement control is a powerful tool for assessing sensory, motor and cognitive function (Ramat, Leigh, Zee, & Optican 2007). The circuitry responsible for the efficient and accurate execution of saccadic eye movements involves multiple cortical and subcortical brain regions, and the roles that these brain regions play in controlling eye movement behaviors have been extensively investigated (Munoz & Everling 2004; Ramat,
Eye movement tasks can be used to characterize deficits in executive functions and motor control in children with FASD (Green, Munoz, Nikkel, & Reynolds 2007; Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009; Paolozza et al. 2013a). In particular, error rates in the antisaccade task (requiring participants to look to the opposite side of a screen when a peripheral target appears) were significantly elevated in children with FASD (Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009; Paolozza, Titman, Brien, Munoz, & Reynolds 2013a). This task requires the suppression of the automatic response to look towards the peripheral target as well as the inversion of the target vector to guide the participant’s voluntary response in the opposite direction (Munoz & Everling 2004), and thus requires extensive sensory-motor remapping to homologous networks ipsilateral to the stimulus (Moon et al. 2007; Zhang & Barash 2000). The memory-guided saccade task requires participants to make saccades to the remembered locations of previously presented stimuli and measures working memory and response inhibition simultaneously. In a previous study (Paolozza, Titman, Brien, Munoz, & Reynolds 2013a), we showed that children with FASD were unable to inhibit the automatic response and looked to the visual targets before receiving the appropriate go signal (timing error). Therefore, voluntary saccades generated by a goal-directed plan are particularly vulnerable to prenatal exposure to alcohol. The current study allows for further investigation into voluntary saccades using a larger sample size and, for the first time, how eye movement behaviours are related to measures of inhibitory control obtained from standardized psychometric tests.

Single neuron recordings in monkeys have revealed that the dorsolateral prefrontal cortex (DeSouza, Menon, & Everling 2003; Funahashi et al. 1993), lateral intraparietal area (Gottlieb &
Goldberg 1999; Schlag-Rey, Amador, Sanchez, & Schlag 1997; Zhang & Barash 2000), frontal eye fields (Everling & Munoz 2000), secondary eye fields (Amador et al. 2004; Schlag-Rey, Amador, Sanchez, & Schlag 1997) and superior colliculus (Everling, Dorris, & Munoz 1998a; Everling, Dorris, Klein, & Munoz 1999) all play a role in oculomotor control. Single cell studies have also found that successful inhibition relies on preparatory activity in these regions (Amador, Schlag-Rey, & Schlag 2004; Everling & Munoz 2000). Functional MRI data have indicated that the anterior cingulate cortex is also important for inhibitory control of eye movements (Velanova et al. 2008). Top-down inhibition of the automatic saccade towards a visual target is required for a successful antisaccade or memory-guided trial which is thought to rely heavily on the dorsolateral prefrontal cortex. This observation is further strengthened with the analysis of patients with discrete dorsolateral prefrontal cortex lesions who have difficulty in suppressing the automatic prosaccade when performing the antisaccade task (Pierrot-Deseilligny et al. 2002). The frontal cortex continues to mature from childhood to adulthood, thus allowing for greater inhibitory control over behaviour as one develops, which is reflected by a decrease in inhibitory errors during the performance of oculomotor tasks (Hikosaka 1997; Hwang et al. 2010; Luna, Thulborn, Munoz, Merriam, Garver, Minshew, Keshavan, Genovese, Eddy, & Sweeney 2001).

There are numerous studies showing inhibition deficits on a variety of psychometric tasks among children with FASD or prenatal alcohol exposure (PAE), even among those as young as 4 years (Mattson, Goodman, Caine, Delis, & Riley 1999; Noland et al. 2003; Rasmussen and Bisanz 2009). For example, children with FASD or PAE have considerable difficulty on Stroop-like inhibition tests especially when interference or switching is involved (Mattson, Goodman, Caine, Delis, & Riley 1999; Rasmussen & Bisanz 2009). Children with FASD are also
impaired on inhibition subtests of the Neuropsychological Assessment (NEPSYII) such as inhibition (switching condition) and response set (Rasmussen, Tamana, Baugh, Andrew, Tough, & Zwaigenbaum 2012). Deficits in inhibition place children with FASD at risk of educational disruptions, developing emotional and behavioural disorders, and many eventually have trouble with the law, and engage in health-threatening behaviours such as substance abuse, smoking, and risky sexual behavior (Streissguth, Bookstein, Barr, Sampson, O'Malley, & Young 2004).

The circuitry responsible for the efficient and accurate execution of psychometric tasks also involves multiple cortical and subcortical brain regions. Evidence from functional neuroimaging, animal models and human lesion studies indicates that the prefrontal cortex plays a vital role in inhibition (Aron and Poldrack 2005; Iversen and Mishkin 1970). Several developmental studies have reported increased frontal activation in children and adolescents compared to adults during psychometric inhibition tasks, interpreted as reflecting more diffuse prefrontal function (Booth et al. 2003; Casey et al. 1997; Durston et al. 2002). Prior neuroimaging studies of individuals with FASD have associated anterior and orbital frontal cortical shape abnormalities in children prenatally exposed to alcohol with inhibitory deficits (Sowell et al. 2002a). For example, Fryer et al. (2007) found increased activation in the prefrontal cortex during the inhibition portion of a go/no-go task, suggesting that greater cognitive resources were required to complete the task. Similarly, a study using event-related potentials found that children with FASD display greater activation in frontal and parietal regions during a go/no-go task indicating increased cognitive effort, despite scoring similar to controls (Burden et al. 2009). The regulation of response inhibition has also been associated with reductions in the volume of the caudate nucleus in children with FASD (Mattson et al. 1996a). Therefore, many of the brain areas utilized for psychometric testing seem to overlap with those used during eye movement.
control tasks.

Adverse effects of prenatal alcohol exposure have been demonstrated using psychometric testing and eye movement control studies; however, these measures have not been empirically linked together in children with FASD. Based on overlapping brain regions believed to be engaged during both psychometric testing and eye movement control tasks, we hypothesized that a relationship should exist between these measures. That is, an increase in the frequency of errors in eye movement control tasks will correspond to lower scores in specific psychometric tests that probe the higher cognitive function of response inhibition. Response inhibition, attention and working memory have been described as aspects of the same mental process and some of the circuitry overlaps, but the neuronal computations are distinct (Miller and Cohen 2001). Therefore, we compared the performance of children with FASD in specific eye movement control tasks to scores in separate tests of attention, working memory and response inhibition. The objective of this study was to determine if a relationship exists between these measures in children with FASD which can better point to specific brain regions damaged by prenatal alcohol exposure.
4.3 Methods

4.3.1 Participants

All experimental procedures were reviewed and approved by the Human Research Ethics Board at Queen’s University, University of Alberta, Children’s Hospital of Eastern Ontario, and University of Manitoba. Informed consent was obtained from the parent before the protocol and the children also completed an assent form. Children with FASD (FAS, pFAS, ARND; n=72) were assessed according to the Canadian Guidelines (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005a), and recruited through referrals from clinicians in diagnostic clinics from Kingston, ON, Ottawa, ON, Edmonton, AB, Cold Lake, AB, and Winnipeg, MB, as part of a larger study funded by NeuroDevNet (Reynolds, Weinberg, Clarren, Beaulieu, Rasmussen, Kobor, Dube, & Goldowitz 2011). Children who had confirmed prenatal alcohol exposure through their respective clinic (PAE; n=21) but did not have a diagnosis of FASD because they do not meet all of the diagnostic criteria were also recruited. Typically developing children (n=139) were recruited from the same geographical areas and were excluded if they had any neurological disorder, psychiatric disorder, or visual disturbance (other than requiring corrective lenses) (Table 4-1). Due to constraints such as age restrictions, quality control, and geographic area not all children completed both psychometric testing and eye movement control experiments. Participants were asked to withhold any medications typically taken on the day of the testing to remove any confounding effects of stimulant drugs, which have relatively short half-lives. Participants received gift cards for the 2-hour test session. Socioeconomic status (SES) was calculated using Hollingshead’s Four-Factor Index of Social Status for the FASD, PAE, and control groups and analyzed for group differences (Hollingshead 1975). Study data
were collected and managed using REDCap electronic data capture tools hosted at Queen’s University (Harris, Taylor, Thielke, Payne, Gonzalez, & Conde 2009).
### Table 4-1 Demographic Information for FASD and PAE group

<table>
<thead>
<tr>
<th>Age</th>
<th>Average (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FASD</td>
<td>11.5±3 years</td>
</tr>
<tr>
<td>PAE</td>
<td>10.9±2 years</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal Alcohol Syndrome</td>
<td>8</td>
</tr>
<tr>
<td>Partial Fetal Alcohol Syndrome</td>
<td>14</td>
</tr>
<tr>
<td>Alcohol Related Neurodevelopmental Disorder</td>
<td>50</td>
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<tr>
<td>Prenatal Alcohol Exposure</td>
<td>21</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Comorbidities</th>
<th>FASD n (% of group)</th>
<th>PAE n (% of group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attention Hyperactivity Deficit Disorder</td>
<td>45 (63)</td>
<td>12 (57)</td>
</tr>
<tr>
<td>Anxiety Disorder</td>
<td>9 (13)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Oppositional Defiant Disorder</td>
<td>10 (14)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Language Processing Disorders</td>
<td>10 (14)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Depression</td>
<td>4 (6)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Other Neurological Disorder</td>
<td>10 (14)</td>
<td>5 (24)</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Medication</th>
<th>FASD n (% of group)</th>
<th>PAE n (% of group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulants</td>
<td>32 (44)</td>
<td>7 (33)</td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>15 (21)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>7 (10)</td>
<td>2 (10)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (4)</td>
<td>5 (24)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other</th>
<th>FASD (average)</th>
<th>PAE (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Socioeconomic Status</td>
<td>41</td>
<td>40.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>FASD n (% of group)</th>
<th>PAE n (% of group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Nations/Metis</td>
<td>44 (61)</td>
<td>18 (86)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>25 (35)</td>
<td>3 (14)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (4)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
4.3.2 The NEPSY, Second Edition (NEPSY-II)

The NEPSY-II is a standardized psychometric test battery for children 3-16 years of age (Korkman, Kirk, & Kemp 2007). This battery assesses multiple domains of executive functioning, memory, sensorimotor functioning, social perception, language, and visuospatial processing. The NEPSY-II yields raw, standardized scores and percentile rankings based on age. Data from three subtests (Auditory Attention, Response Set, and Inhibition subtests) were used in this study. Each subtest used included a teaching example and practice round where the experimenter had the opportunity to ensure the child understood the instructions and correct the child’s mistakes. All subtests have a mean standard score of 10 with a standard deviation of 3. Performance of these tests improves with age, which is reflected by an age-dependent increase in raw scores obtained in the tasks. Standard scores are age-corrected, and once age was removed by using the standard scores the controls all fluctuated around the mean (10) for the tasks, as expected for a random population of typically developing control children.

4.3.2.1 Auditory Attention

Auditory Attention assesses auditory selective and sustained attention. The child is instructed to point to one specific stimulus when a specific word is heard on an audio recording (i.e. point to the red circle when only the word “red” is heard). The audio recording is a string of random words read out (1 word per second). Three types of errors are scored for this task. A commission error occurs when the child provides an incorrect response. An omission error occurs when the child fails to provide the correct response. An inhibitory error occurs when the child responds to a colour word by touching that colour when it is not the correct response.
4.3.2.2 Response Set

Response Set assesses auditory selective and sustained attention with new rules that require shifting and inhibition of the previously learned rules on the Auditory Attention test. The child is instructed to point to one specific stimulus when a specific word is heard on an audio recording (i.e. point to the red circle when the word “yellow” is heard). The same three types of errors as Auditory Attention are scored for this task.

4.3.2.3 Inhibition

The inhibition subtest has three conditions: 1) Naming requires the child to name the shape or say the direction of the arrow for a full page of shapes or arrows, which measures attention; 2) Inhibition requires the child to provide the opposite of the correct response (i.e. say circle when see square or say up when arrow points down), measuring inhibition; and 3) Switching requires the child to switch between the correct response and the opposite response depending on the color of the shape or arrow (i.e. if the shape is black then say the correct response and if the shape is white say the opposite response) and measures the ability to switch between the automatic and inhibitory response types. An error occurs for all three conditions when the child provides an incorrect response. It is also recorded if the child self corrects this error.

4.3.2.4 Data Analysis of Psychometric Measures

Differences between groups were analyzed using a one-way analysis of variance (ANOVA) coupled with Newman-Keuls Test for Multiple Comparisons. Effect sizes were also calculated for the dependent variables using Cohen’s $d$ scores. Data are expressed as mean ± s.e.m. for children in the FASD (n=72; average age 11.5±3 years, 38 males), control (n=90; average age 10.0±3 years, 44 males) and PAE (n=21; average age 10.9±3 years, 10 males)
groups, which were matched as closely as possible for age and gender. Standard scores of the psychometric measures were used for all analyses. The combined scaled scores for all subtests were used for analysis because these are calculated based on both correct and error trials.

IQ was not obtained from any of the participants in this study, since IQ has not been consistently shown to affect performance on psychometric tests (Dennis et al. 2009), and matching groups on IQ can result in mismatching on other important variables (Stigler and Miller 1993). More specifically, studies with children and adolescents with FASD have shown that executive functioning deficits remain even after controlling for IQ (Olson, Feldman, Streissguth, Sampson, & Bookstein 1998), and that controlling for IQ yields little change in the outcome (Quattlebaum and O’Connor 2012).

4.3.3 Saccadic eye movement recordings

Participants were seated comfortably in a dark, quiet room on a stable chair. Eye position was recorded using the Eyelink 1000 (SR Research, Kanata, ON). The 17” LCD monitor and mounted infrared camera were at a distance of 58-64 cm from the left eye. The position of the left pupil was digitized in both the vertical and horizontal axes at a sampling rate of 500 Hz. Performance was assessed in two tasks: the antisaccade task and then a memory-guided saccade task. Before each task the eye movements of each participant were calibrated using nine on-screen targets (eight around the periphery and one central). The targets were flashed sequentially around the screen and the participant fixated on each one. After calibration, the process was repeated to validate that the average error between fixation and target was less than 2 degrees and that no loss of eye tracking occurred. This also ensured that the participants had no visual disturbances.
4.3.3.1 Behavioral Tasks

In the antisaccade task, each trial started with illumination of a central fixation point (FP) for 800-1200ms. The FP then disappeared and, after a delay of 200ms (gap period), a peripheral target appeared randomly at 10° to the left or right of the central FP. Participants were given 1000ms to initiate and complete a saccade to the correct location and were instructed to look away from the target, towards the opposite side of the screen. No error feedback was given. One block of 60 antisaccade trials was obtained from each participant.

In the memory-guided saccade task, participants were instructed to maintain fixation at the central FP while two peripheral targets appeared. After the FP disappeared, they were required to make a saccade to the remembered locations of the peripheral targets. The screen was divided into four quadrants in which the peripheral targets could appear. Each quadrant consisted of nine potential target locations in an invisible 3 by 3 grid centered at a 10° visual angle from the FP. The FP was illuminated for 200-1000ms before the appearance of the two targets. The two targets then appeared briefly in immediate succession for 100ms each, within two of the four quadrants of the screen. Participants were required to fixate on the central FP for an additional random time of 0, 600, 1200, or 1800ms between the disappearance of the second peripheral target and the disappearance of the FP. They were instructed to remember the spatial location and order of the peripheral targets, and to make two saccades as accurately as possible to these locations in the same sequence but only after the disappearance of the FP. A single block of 72 trials was collected for this task.

4.3.3.2 Data Analysis of Eye Tracking Measures

Data were analyzed using custom software developed in MATLAB (R2009b, The Mathworks, Inc, Natick, Massachusetts). Saccades were defined as having a speed of greater
than 2.5 times the standard deviation of the background noise (measured during fixation) for at least five sample points. Only trials where the participant was fixating on the FP at the appropriate time were used. If the participant broke fixation inappropriately (i.e. not to a target location or away from the screen) the trial was discarded from analysis. Any trials where eye tracking was lost were removed. To be included in the analysis, each participant had to achieve greater than 50% viable trials in each of the tasks. The data for one control participant and two FASD participants were excluded due to less than 50% viable trials.

Due to the large control sample size obtained in this study we were able to standardize the eye movement control scores by age. For both saccadic eye movement tasks, there was a significant improvement in task performance with increasing age. Therefore, the data were age corrected by calculating a standard score (t-score) for each age. Scores for the FASD and PAE groups were then calculated using the age-dependent t-score equation obtained from the control group. Differences between groups were analyzed using a one-way ANOVA coupled with Newman-Keuls Multiple Comparison Test. Effect sizes were also calculated for the dependent variables using Cohen’s $d$ scores. Data are expressed as mean ± s.e.m. for children in the FASD (n=66; 11±3 average age years, 35 males), control (n=105; 10±3 average age years, 50 males) and PAE (n=14; 10±2 average age years, 5 males) groups, which were matched as closely as possible for age and sex.

Saccade measures for all viable trials for the two tasks were assessed by examining the inhibition outcome measures. Direction errors in the antisaccade task were defined as any initial saccade in the wrong direction with respect to the instruction (i.e., towards the target). In the memory-guided task, individual trials were assigned as either correct, timing errors (saccades initiated before 90ms after the go signal), and/or sequence errors (initial saccade made closer to
the second peripheral target location than to the first target, therefore in the wrong sequence).

These measures assessed spatial working memory, response inhibition and attention, and were examined because they are frequently reported as deficits in children with FASD (Kodituwakku 2009; Mattson, Goodman, Caine, Delis, & Riley 1999; Rasmussen & Bisanz 2009).

4.3.4 Correlation Analysis

Hypothesis-driven Pearson correlations were used to identify whether psychometric test scores were associated with eye movement control measures. The scores from each measure were chosen if the FASD group was significantly different from controls. Bonferroni correction for multiple comparisons was applied to the correlational analyses. Children in the FASD/PAE (n=78; average age 11±3 years, 39 males) and control (n=57; average age 11±3 years, 28 males) groups, who completed both psychometric testing and eye movement tasks were matched as closely as possible for age and gender. The FASD group and the PAE group were combined for this analysis to increase the statistical power and because they did not differ from one another on most measures.
4.4 Results

4.4.1 Demographic Factors

To investigate the possible role of demographic variables we performed several analyses on comorbidities, SES, group subtypes, and ethnicity. Children with FASD with or without a comorbid disorder of ADHD were compared on all measures presented by first visually examining the data, followed by t-tests between those with or without a comorbid disorder of ADHD. In agreement with a previous study (Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009a), no differences were found. Due to the low frequency of other comorbid disorders we were only able to investigate ADHD. We next performed ANOVAs to examine subgroup differences by dividing the FASD group into two subgroups (FAS/pFAS and ARND), which were then compared to each other and to the control and PAE group. No group differences were found between the two FASD groups (FAS/pFAS and ARND) on any psychometric or eye tracking measure. SES was examined for group differences using an ANOVA and the control group was found to have a significantly higher status than both the FASD and PAE groups (F(2,173)= 6.7, p=0.0015). However, SES showed no significant relationship to any of the psychometric or eye movement measures in any group. This was analyzed by both visually examining the data and subsequently running Pearson’s correlations.

Ethnicity was also analyzed since the majority of the control group (96%) was Caucasian and the majority of our FASD group was First Nations (61%). This was accomplished by dividing the FASD group into those who are identified as First Nations and those with any other ethnicity (primarily Caucasian). The data were then examined visually to look for any trending disparities, and then t-tests were run to look for significant differences. There were no significant differences on any of the psychometric and eye movement measures between the two ethnic groupings.
represented among the FASD participants. Therefore, comorbidities, diagnostic subgroup, SES, and ethnicity did not influence the data and did not need to be included as covariates.

4.4.2 Psychometric Testing

The FASD group achieved test scores that were significantly lower than controls on Auditory Attention, Response Set, and Inhibition subtests from the NEPSY-II (see Table 4-2). The FASD group was significantly lower than both the control group and the PAE group on Auditory Attention (F(2,183) = 21.0, p<0.0001) (Fig. 4-1A). The one-way ANOVA revealed significance for Response Set (F(2,162) = 7.1, p=0.001) (Fig. 4-1B); however, in contrast to Auditory Attention, both the FASD and PAE groups were lower than the control group but were not different from each other.

The one-way ANOVA was significant for the Naming condition of the Inhibition subtest (F(2,182) = 14.1, p<0.0001) (Fig. 4-1C); the FASD and PAE groups were both different from the control group, but not from each other. Similarly, there was a significant effect of group on the Inhibition condition of the Inhibition subtest (F(2,182) = 29.2, p<0.0001) (Fig. 4-1D); the FASD group was significantly lower than both the control group and the PAE groups. Finally, the one-way ANOVA revealed a significant effect of group in the Switching condition of the Inhibition subtest (F(2,156)=31.9, p<0.0001) (Fig. 4-1E); all three groups were significantly different from each other.
<table>
<thead>
<tr>
<th>Test</th>
<th>FASD (mean±SEM)</th>
<th>PAE (mean±SEM)</th>
<th>Con (mean±SEM)</th>
<th>Con vs. FASD (Cohen’s $d$)</th>
<th>Con vs. PAE (Cohen’s $d$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Auditory Attention</td>
<td>7.5±0.4</td>
<td>9.4±0.7</td>
<td>10.8±0.3</td>
<td>1.0</td>
<td>not sign</td>
</tr>
<tr>
<td>Response Set</td>
<td>9.6±0.4</td>
<td>10.0±0.6</td>
<td>11.4±0.3</td>
<td>0.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Inhibition: Naming</td>
<td>6.6±0.4</td>
<td>7.9±0.8</td>
<td>9.6±0.3</td>
<td>0.8</td>
<td>0.49</td>
</tr>
<tr>
<td>Inhibition: Inhibition</td>
<td>6.2±0.4</td>
<td>8.9±0.7</td>
<td>10.1±0.4</td>
<td>1.2</td>
<td>not sign</td>
</tr>
<tr>
<td>Inhibition: Switching</td>
<td>6.7±0.4</td>
<td>8.6±0.7</td>
<td>10.6±0.3</td>
<td>1.4</td>
<td>0.73</td>
</tr>
<tr>
<td>Direction Errors</td>
<td>55.4±5.8</td>
<td>50.2±5.9</td>
<td>50.0±3.1</td>
<td>-0.5</td>
<td>not sign</td>
</tr>
<tr>
<td>Timing Errors</td>
<td>60.5±5.8</td>
<td>56.9±7.8</td>
<td>50.0±3.2</td>
<td>-0.7</td>
<td>not sign</td>
</tr>
</tbody>
</table>
Figure 4-1 Attention and inhibition measures from the NEPSY-II. Data are mean ± s.e.m. for subjects in the control group (n=90) shown in blue, FASD group (n=72) shown in red and the PAE group (n=21) shown in purple. A: Auditory Attention subtest. B: Response Set subtest. C: Naming condition of the Inhibition subtest. D: Inhibition condition of the Inhibition subtest. E: Switching condition of the Inhibition subtest. *p<0.05, **p<0.01, ***p<0.001.
4.4.3 Eye Movement Control

Children with FASD performed significantly worse than controls on the antisaccade and memory-guided saccade tasks (see Table 4-2). The one-way ANOVA revealed a significant effect of group for direction errors in the antisaccade task ($F(2,181) = 5.1, p=0.007$) (Fig. 4-2A); the FASD group made significantly more errors than the control, but not the PAE group. The FASD and PAE groups corrected these direction errors on average 86% of the time, thereby demonstrating that they understood the task. Similarly, the one-way ANOVA revealed a significant effect for timing errors in the memory-guided saccade task ($F(2,158) = 10.26, p<0.0001$) (Fig. 4-2B); the FASD group made more errors than the control group, whereas the difference between the control and PAE groups approached significance ($p=0.028$, adjusted for multiple comparisons).
Figure 4-2 Inhibition measures obtained from eye movement control tasks. Data are mean ± s.e.m. for subjects in the control group (n=105) shown in blue, FASD group (n=66) shown in red, and the PAE group (n=14) shown in purple. A: Direction errors in antisaccade task. B: Timing errors in memory-guided saccade task. **p<0.01, ***p<0.001.
4.4.4 Correlations

After correcting for age, there was no correlation between direction errors and the Inhibition and Switching conditions among the control group (Fig. 4-3); the FASD/PAE group did however show a negative correlation for these same relationships (Fig. 4-3). None of the NEPSY-II subtests were significantly correlated with timing errors from the memory-guided saccade task.
Figure 4-3 Correlation of direction errors and Inhibition subtests. Data are individual data points for subjects in the control (n=57), and combined FASD and PAE (n=78) groups. Correlation of control group direction errors and Inhibition (A) or Switching (C) conditions of the Inhibition subtest. Correlation of FASD/PAE group direction errors and Inhibition (B) or Switching (D) conditions of the Inhibition subtest.
Discussion

4.4.5 General Findings

The objective of this study was to compare the performance of children with FASD and PAE to healthy controls and determine if a performance-based relationship exists between psychometric tests and eye movement control tasks in children with FASD and PAE. Children with FASD exhibited deficits in response inhibition as seen in the response set subtest and the inhibition and switching conditions of the inhibition subtest. Problems in attention among the FASD group were also seen on the auditory attention subtest and the naming condition of the inhibition subtest. The eye movement control tasks reveal that children with FASD show problems in response inhibition as they made a greater number of errors on the antisaccade and memory-guided tasks. Among the FASD/PAE group (when combined), both the inhibition and switching conditions, but not the naming condition, were correlated with direction errors in the antisaccade task. This result suggests that common brain structures are indeed important for regulating inhibitory control in both sets of tasks, and that brain injury induced by prenatal alcohol exposure results in poor performance on both psychometric and eye movement control measures. However, there were no correlations among the control group. This is expected since the effect of age, the major contributing factor to task performance in typically developing children, was removed by the use of age-corrected standard scores. The fact that a significant relationship was still present in the FASD/PAE group indicates that something other than age is driving this correlation. We hypothesize that this additional factor is the brain injury induced by prenatal alcohol exposure (Hwang, Velanova, & Luna 2010; Korkman et al. 2012; Luna et al. 2001). That is, in healthy controls, the age-corrected performance on both tests of inhibition fluctuates around the mean for all ages, and therefore no correlation between these outcome
measures should exist. There were no correlations between psychometric tests and timing errors on the memory-guided task for either the FASD/PAE or control groups probably due to the multiple cognitive domains required for the task. Thus, this pattern of results indicates that response inhibition deficits in the psychometric testing are associated with difficulty controlling automatic eye movements in those prenatally exposed to alcohol.

4.4.6 Psychometric Testing

Children with FASD are often referred for diagnostic assessment because of problems with executive functions including inhibition, attention and working memory. Children with FASD display deficits on psychometric tests of inhibition, particularly when interference or switching is involved (Mattson, Goodman, Caine, Delis, & Riley 1999; Rasmussen & Bisanz 2009). In the current study, children with FASD showed similar magnitude of response inhibition problems on all tasks. The children in the PAE group, who are presumably less alcohol affected, exhibited intermediate scores between the FASD and control groups on some tests, but seemed to perform significantly worse than controls on the more difficult tasks involving interference and switching. Specifically, the PAE group was not significantly different from controls on Auditory Attention but was on Response Set, which requires additional instructions and switching. A similar pattern was seen in the Inhibition subtest; children in the PAE group were not significantly different from controls on the Inhibition condition but were significantly different on the Switching condition and Naming condition. These results may indicate that children with a diagnosis of FASD show deficits in attention and response inhibition across all levels of difficulty but those with PAE, who did not receive a diagnosis, only demonstrate deficits once tasks are sufficiently complex, and include shifting and interference demands.

Previous literature has linked psychometric response inhibition deficits to many different
areas in the brain, particularly the frontal cortex. Fryer et al. (2007) linked inhibition to the prefrontal cortex in healthy controls showing increased activation during the inhibition portion of a go/no-go task. In other words, the healthy brain focuses greater cognitive resources in the prefrontal cortex to successfully inhibit a response (Fryer et al. 2007). A combination event-related potential-functional magnetic resonance imaging study in healthy controls found that the anterior cingulate and the frontoparietal control network activity are active during no-go trials (Jamadar et al. 2010). A different study using event-related potentials found that children with FASD display an even greater activation in these same regions compared to controls, indicating that they require increased cognitive effort to correctly inhibit a response (Burden, Andrew, Saint-Amour, Meintjes, Molteno, Hoyme, Robinson, Khaole, Nelson, Jacobson, & Jacobson 2009). The regulation of response inhibition has also been associated with reduced volume in the caudate nucleus in children with FASD (Mattson et al. 1996). Therefore, it is likely that damage to these areas is contributing to the deficit in response inhibition seen during the psychometric testing.

4.4.7 Eye Movement Control

Eye movements are controlled by both overlapping and distinct brain areas compared with psychometric testing. Lesions in the prefrontal cortex lead to an increase in direction errors, while lesions to the frontal eye fields, a region that is presumably not involved in psychometric testing, causes prolonged reaction times (Pierrot-Deseilligny, Ploner, Muri, Gaymard, & Rivaud-Pechoux 2002). Structural imaging studies of children with prenatal alcohol exposure have found cortical thickness abnormalities over large areas of dorsolateral prefrontal lobes, predominantly in the right hemisphere (Sowell et al. 2008b). The basal ganglia are also important for response inhibition during eye movement tasks (Munoz & Everling 2004). Marked volume reductions and
decreased metabolic rates have been found in the basal ganglia in children with FASD (Clark et al. 2000; Lebel et al. 2011). The anterior cingulate cortex is the final overlapping region important for inhibitory control of eye movements as it shows deactivation during successful inhibition of the automatic prosaccade. Additionally, the dorsal anterior cingulate cortex shows greater activity when a direction error is made, and the activity increases from childhood to adulthood indicating that the dorsal anterior cingulate cortex plays an important role in performance enhancement through successful maturation (Luna, Velanova, & Geier 2008). The supramarginal gyrus, an area in the inferior parietal lobe, plays a large role in response inhibition in the antisaccade task (Ettinger et al. 2008). Volume reductions in the FASD population have been seen across the entire cortex but the parietal lobe appears to be an area particularly vulnerable to these reductions (Archibald et al. 2001; Sowell et al. 2002b). The current study adds to the literature describing the effects of prenatal alcohol exposure on clinical and functional impairments in areas of the brain vital to proper response inhibition by linking similar brain regions vital to both psychometric testing and eye movement control.

Similar to the antisaccade task, the delayed memory-guided task assesses the control of internally-guided saccades. In the present study, the FASD group demonstrated increased timing errors in the delayed memory-guided task, reflecting deficits in either the ability to suppress and/or to inhibit saccadic responses. Previous studies have revealed increased timing errors in the ADHD, Huntington’s disease and Parkinson’s disease populations, implicating the frontostriatal circuitry in the successful inhibition of responses during a delay period (Chan et al. 2005; Mostofsky et al. 2001; Pelsch et al. 2008). The difference between this task and the antisaccade task is that participants must use previously presented sensory information to plan and initiate a motor response at the appropriate time. This task, therefore, requires the integration
of multiple domains of cognitive function, including spatial working memory and response suppression, and also requires the participant to simultaneously remember two instructions. None of the psychometric tests correlated to the memory-guided task probably because of the above reasons.

4.4.8 Domains of Executive Functioning

The psychometric and eye movement control tasks probe three main domains of executive functioning: response inhibition, working memory, and attention, which are thought to be largely overlapping when completing tasks. Miller and Cohen (2001) argue that these functions rely on the prefrontal cortex which represents goals and rules in the form of patterns of activity. These patterns allow attention to have a biasing effect favoring the task-relevant stimulus, and inhibition acts to suppress distractions from the irrelevant information (Miller & Cohen 2001). Structural imaging studies have found decreases in prefrontal cortex volume in those with FASD (Archibald, Fennema-Notestine, Gamst, Riley, Mattson, & Jernigan 2001). Therefore, we see an overall deficit in both inhibition and attention; however, only the antisaccade task and the inhibition task measure inhibition directly and therefore were significantly correlated. In contrast, auditory attention and response set were not related perhaps because these tasks focus more on attention and require the comprehension and maintenance of multiple instructions. The prefrontal cortex is thought to play a facilitating role to intermediate systems in working memory representing a distinct pathway in the brain. This provides a further explanation to why the memory-guided task does not directly relate to the inhibition tasks (Miller & Cohen 2001). By examining each of these tasks independently we can be more confident that this relationship involves isolated inhibition pathways and not attention or working memory pathways.
4.5 Conclusions

The results of this study have important clinical implications. As previously stated children with FASD with or without a comorbid disorder of ADHD were not significantly different on any of the measures used. While we did not directly compare participants with ADHD and FASD in the current study, a previous study that utilized a novel eye movement paradigm did find differences in bottom-up processing between these two clinical populations that served to distinguish the two groups (Tseng et al. 2013). Future studies need to be conducted to better understand the similarities and differences between these two disorders. The psychometric tests and eye movement tasks point to specific clinical demonstrations of brain areas affected in children with FASD. While we cannot exclude the possibility that other co-morbid neurological or psychiatric disorders in our study population play a role in the observed outcomes, response inhibition deficits are not a prominent feature of these disorders, and the frequency of these disorders is too low to definitely answer this question. The large effect sizes seen in the psychometric testing do support the use of the NEPSY-II as a useful diagnostic tool in the FASD population.

The procedure for FASD diagnosis requires specialized training, a very large time commitment, may not be accessible to the rural population, and the cost is considerable. Thus, effective screening tools could be the first step to identify those who need to be referred for the full assessment. This study indicates that deficits in eye movement control may be an effective screening assessment tool in clinic because it successfully differentiates children with prenatal alcohol exposure from controls. Additionally, the eye movement control tasks directly relate to the psychometric tests used during diagnosis and may help with early identification of children who may benefit from a multidisciplinary diagnostic assessment due to overlapping brain
structures that are damaged as a result of prenatal alcohol exposure. Eye tracking is portable, does not need to be administered by clinicians, and one session takes less than an hour making it a desirable choice as a screening tool. It identifies individuals with deficits in response inhibition and can be used to recognize those in need of immediate interventions which can be put in place while the individual is awaiting the full assessment. This could then lead to earlier interventions and potentially less adverse outcomes for those prenatally exposed to alcohol (Streissguth, Bookstein, Barr, Sampson, O'Malley, & Young 2004). In addition, the more expensive and time-consuming multidisciplinary team assessment for FASD would then be available for the targeted population, creating more availability and cost-effectiveness for assessment of FASD.
4.6 Acknowledgements

We thank the participants and their families for taking part in the study. This work was supported by NeuroDevNet, which is funded by the Networks of Centres of Excellence, a program of the federal government to advance science and technology. We also acknowledge the NeuroDevNet Neuroinformatics Core for data management system implementation and support and Donald Brien for his technical expertise in the collection and analysis of the eye movement data.
Chapter 5

Working memory and visuospatial deficits correlate with oculomotor control in children with Fetal Alcohol Spectrum Disorder

5.1 Abstract

Previous studies have demonstrated that children with Fetal Alcohol Spectrum Disorder (FASD) exhibit deficits in measures of eye movement control that probe aspects of visuospatial processing and working memory. The goal of the present study was to examine, in a large cohort of children with FASD, prenatal alcohol exposure (PAE) but not FASD, and typically developing control children, the relationship between performance in eye movement tasks and standardized psychometric tests that assess visuospatial processing and working memory. Participants for this dataset were drawn from a large, multi-site investigation, and included children and adolescents aged 5-17 years diagnosed with an FASD (n=71), those with PAE but no clinical FASD diagnosis (n=20), and typically developing controls (n=111). Participants completed a neurobehavioral test battery and a series of saccadic eye movement tasks. The FASD group performed worse than controls on the psychometric and eye movement measures of working memory and visuospatial skills. Within the FASD group, Digit Recall, Block Recall, and Animal Sorting were negatively correlated with sequence errors on the memory-guided task, and Arrows was negatively correlated with prosaccade endpoint error. There were no significant correlations in the control group. These data suggest that psychometric tests and eye movement control tasks may assess similar domains of cognitive function, and these assessment tools may be measuring overlapping brain regions damaged due to prenatal alcohol exposure. The results of
this study demonstrate that eye movement control tasks directly relate to outcome measures obtained with psychometric tests and are able to assess multiple domains of cognition simultaneously, thereby allowing for an efficient and accurate assessment.
5.2 Introduction

The full spectrum of adverse effects induced by prenatal alcohol exposure, which includes several diagnostic subgroups, is collectively referred to as Fetal Alcohol Spectrum Disorder (FASD) (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). FASD is estimated to occur in at least 1% of the population in North America (May, Gossage, Kalberg, Robinson, Buckley, Manning, & Hoyme 2009). The developing brain is the principal target organ for gestational alcohol exposure and this type of injury can manifest as intellectual, neurological and behavioural abnormalities. These abnormalities can produce a wide variety of mild to severe brain dysfunctions in processes such as learning and memory, executive function, social communication, attention, and sensory-motor skills (Kodituwakku 2009; Mattson, Crocker, & Nguyen 2011; Rasmussen 2005), which may contribute to the negative behavioural, neuropsychiatric, and maladaptive outcomes commonly observed in this population (Rasmussen, Andrew, Zwaigenbaum, & Tough 2008). This study focused on working memory and visuospatial deficits, which are prevalent problems for those affected by FASD. Poor working memory and/or visuospatial skills in children are associated with poor academic performance especially in reading and mathematics (Alloway et al. 2009), and thus it is likely that these same deficits contribute to poor academic outcomes in children with FASD.

Saccades are rapid eye movements that bring new visual targets onto the fovea of the retina. The circuitry responsible for the efficient and accurate execution of saccadic eye movements involves multiple cortical and subcortical brain regions, and the roles that these brain regions play in controlling eye movement behaviors have been extensively investigated (Munoz & Everling 2004; Ramat, Leigh, Zee, & Optican 2007; Scudder, Kaneko, & Fuchs 2002). Measurement of eye movement control is a powerful tool for assessing sensory, motor and
cognitive function in healthy participants, as well as multiple diseases and disorders (Ramat, Leigh, Zee, & Optican 2007), including children with FASD (Green, Munoz, Nikkel, & Reynolds 2007; Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009; Paolozza, Titman, Brien, Munoz, & Reynolds 2013). In particular, children with FASD have been shown to have poor saccade accuracy both when looking to visual targets and when making saccades to the remembered locations of previously presented targets (Paolozza, Titman, Brien, Munoz, & Reynolds 2013).

Working memory is the ability to temporarily store and manipulate information to complete complex tasks such as language comprehension, learning, and reasoning. According to Baddeley’s revised model, working memory consists of four elements: the central executive is responsible for the control of attention and is necessary for preserved immediate recall and integration of information; the phonological loop provides temporary storage of verbal information; the visuospatial sketchpad provides temporary storage of visual and spatial representations; and the episodic buffer is responsible for integrating different types of information together (Baddeley 2000). Previous research has found that children with prenatal alcohol exposure show deficits on tests that assess the function of the central executive, phonological loop, and visuospatial sketchpad (Jacobson, Jacobson, Sokol, & Ager, Jr. 1998; Rasmussen and Bisanz 2011; Streissguth et al. 1990; Uecker and Nadel 1996).

Visuospatial ability involves a wide variety of skills that can entail visual working memory. These visuospatial abilities include processes such as spatial orientation (imagining how an image will appear from another perspective), spatial visualization (manipulating and encoding spatial forms), figural flexibility (imaging a variety of ways to involve a spatial problem), closure speed (initiating an apparent disparate perception into a unified concept), and
reference memory (assesses spatial memory) (Carroll 1993). Children with FASD have been reported to exhibit poorer visuospatial skills when compared to controls on a variety of tasks (Korkman, Kettunen, & Autti-Ramo 2003; Pei et al. 2011b; Quattlebaum and O'Connor 2012; Rasmussen, Tamana, Baugh, Andrew, Tough, & Zwaigenbaum 2012).

Previously, we have shown that visuospatial processing and working memory are impaired on eye movement control tasks in children with FASD (Paolozza, Titman, Brien, Munoz, & Reynolds 2013). The aim of this study was to examine these tasks in a larger sample of children with FASD as well as those with PAE but not FASD. In a previous study, we showed that measures of response inhibition in children with prenatal alcohol exposure obtained from standardized psychometric tests are correlated to measures of response inhibition obtained from eye movement control tasks (Paolozza et al. 2013). Saccadic eye movement tasks can be designed to assess multiple domains of CNS function (sensory, motor, cognitive) simultaneously. Given the extensive knowledge concerning the neural circuitry underlying eye movement control (Munoz & Everling 2004; Ramat, Leigh, Zee, & Optican 2007; Scudder, Kaneko, & Fuchs 2002), examination of the domains of CNS function that are impaired during the performance of eye movement control tasks may be used to help identify brain regions that have been injured by prenatal alcohol exposure. Based on overlapping brain areas believed to be engaged during tests of working memory and visuospatial processing and eye movement control tasks, we hypothesized that a relationship should exist between these measures. More specifically, we hypothesized that in children with FASD the frequency of sequence errors, a measure of working memory, in the memory-guided saccade task will correspond to lower scores in specific psychometric tests that also probe working memory. Additionally, we predicted that an increase in the saccade endpoint error, a measure of visuospatial processing, in the prosaccade task will
correspond to lower scores in psychometric tests that also assess visuospatial processing skills. Therefore, we correlated the performance of children with FASD in specific eye movement control tasks to scores in separate tests of working memory and visuospatial skills.
5.3 Methods

5.3.1 Participants

All experimental procedures were reviewed and approved by the Human Research Ethics Board at Queen’s University, University of Alberta, Children’s Hospital of Eastern Ontario, and University of Manitoba. Informed consent was obtained from the parent before the protocol, and the children also completed an assent form. Children with FASD (FAS, pFAS, ARND; n=71) were assessed according to the Canadian Guidelines (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005) and recruited through referrals from clinicians in diagnostic clinics from Kingston, ON, Ottawa, ON, Edmonton, AB, Cold Lake, AB, and Winnipeg, MB, as part of a large, multi-site study (Reynolds, Weinberg, Clarren, Beaulieu, Rasmussen, Kobor, Dube, & Goldowitz 2011). Children who had confirmed prenatal alcohol exposure from a credible source (PAE; n=20) but did not have a diagnosis of FASD because they do not meet all of the diagnostic criteria were also recruited. Typically developing children (n=111) were recruited from the same geographical areas and were excluded if they had any neurological or psychiatric disorder, or visual disturbance, other than requiring corrective lenses. All groups were matched as closely as possible for age and sex. Due to constraints such as age restrictions, quality control, and geographic area not all children completed both psychometric testing and eye movement control experiments. Participants were asked to withhold any medications (Table 5-1) typically taken on the day of testing to minimize any confounding effects of stimulant drugs, which have relatively short half-lives. Socioeconomic status (SES) was calculated using Hollingshead’s Four-Factor Index of Social Status for the FASD, PAE, and control groups and analyzed for group differences (Hollingshead 1975). Participants received gift cards for the 2-hour test session. We have previously reported data from this cohort showing correlations between
measures of response inhibition obtained from eye movement control tasks and psychometric tests (Paolozza, Rasmussen, Pei, Hanlon-Dearman, Nikkel, Andrew, McFarlane, Samdup, & Reynolds 2014a).
Table 5-1 Demographic information for control, FASD and PAE group

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<th>n</th>
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<tr>
<td>Partial Fetal Alcohol Syndrome</td>
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</tr>
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<td>Alcohol Related Neurodevelopmental Disorder</td>
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<td>Prenatal Alcohol Exposure</td>
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<table>
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<tr>
<th>Comorbidities</th>
<th>FASD n (%)</th>
<th>PAE n (%)</th>
<th>Control n (%)</th>
<th>Chi-Square (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD</td>
<td>45 (63)*</td>
<td>12 (57)*</td>
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<td>0.0009</td>
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<th>PAE n (%)</th>
<th>Control n (%)</th>
<th>Chi Square (p-value)</th>
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<td>Stimulants</td>
<td>32 (44)*</td>
<td>7 (33)*</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Antipsychotics</td>
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<td>Antidepressants</td>
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<td>2 (10)*</td>
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<td>0.0038</td>
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<tr>
<td>Other</td>
<td>3 (4) †</td>
<td>5 (24)*</td>
<td>0 (0)</td>
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</table>

<table>
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<tr>
<th>Other</th>
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<th>PAE (average±SD)</th>
<th>Control (average±SD)</th>
<th>ANOVA (p-value)</th>
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<td>10.1±3 years</td>
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<tr>
<td>Socioeconomic Status</td>
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<td>40.8±11*</td>
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<th>PAE n (%)</th>
<th>Control n (%)</th>
<th>Chi Square (p-value)</th>
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</thead>
<tbody>
<tr>
<td>First Nations/Metis</td>
<td>44 (61)*†</td>
<td>18 (86)*</td>
<td>1 (1)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Caucasian</td>
<td>25 (35)*</td>
<td>3 (14)*</td>
<td>106 (96)</td>
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<tr>
<td>Other</td>
<td>3 (4)</td>
<td>0 (0)</td>
<td>4 (3)</td>
<td>ns</td>
</tr>
</tbody>
</table>

*p<0.05 compared to controls using Fisher’s exact or Tukey’s post-test (where appropriate); †p<0.05 compared to PAE group using Fisher’s exact or Tukey’s post-test (where appropriate)
5.3.2 Saccadic eye movement recordings

Participants were seated comfortably in a dark, quiet room on a stable chair. Eye position was recorded using the Eyelink 1000 (SR Research, Kanata, ON). The 17” LCD monitor and mounted infrared camera were at a distance of 58-64 cm from the left eye. The position of the left pupil was digitized in both the vertical and horizontal axes at a sampling rate of 500 Hz. Performance was assessed in three tasks: the prosaccade, antisaccade and the memory-guided saccade tasks. Before each task the eye movements of each participant were calibrated using nine on screen targets (eight around the periphery and one central). The targets were flashed sequentially around the screen and the participant fixated on each one. After calibration, the process was repeated to validate that the average error between fixation and target was less than 2 degrees and that no loss of eye tracking occurred. This also ensured that the participants had no visual disturbances (e.g., nystagmus, diplopia) that would impair task performance.

5.3.2.1 Behavioral Tasks

In the prosaccade and antisaccade tasks, each trial started with illumination of a central fixation point (FP) for 800-1200ms. The FP then disappeared and, after a delay of 200ms (gap period), a peripheral target appeared randomly at 10° to the left or right of the central FP. Participants were given 1000ms to initiate and complete a saccade to the correct location and were instructed to look towards the target (prosaccade) or away from the target (antisaccade). No error feedback was given. One block each of 60 prosaccade and 60 antisaccade trials was obtained from each participant.

In the memory-guided saccade task, participants were instructed to maintain fixation at the central FP while two peripheral targets appeared sequentially, and after the FP disappeared, they were required to make sequential saccades to the remembered locations of the peripheral
targets in the same order they originally appeared. The screen was divided into four quadrants in which the peripheral targets could appear. Each quadrant consisted of nine potential target locations in an invisible 3 by 3 grid centered at a 10° visual angle from the FP. The FP was illuminated for 200-1000ms before the appearance of the two targets. The two targets then appeared briefly in immediate succession for 100ms each, within two of the four quadrants of the screen. Participants were required to fixate on a central FP for an additional random time of 0, 600, 1200, or 1800ms between the disappearance of the second peripheral target and the disappearance of the FP. They were instructed to remember the spatial location and the order of the peripheral targets, and to make two saccades as accurately as possible to these locations in the same sequence but only after the disappearance of the FP. A single block of 72 trials was collected for this task.

5.3.2.2 Data Analysis of Eye Tracking Measures

Data were analyzed using custom software developed in MATLAB (R2009b, The Mathworks, Inc, Natick, Massachusetts). Saccades were defined as having a speed of greater than 2.5 times the standard deviation of the background noise (measured during fixation) for at least five sample points. Only trials where the participant was fixating on the FP at the appropriate time were used. If the participant broke fixation inappropriately (i.e. not to a target location or away from the screen) the trial was discarded from analysis. Any trials where eye tracking was lost were removed. To be included in the analysis, each participant had to achieve greater than 50% viable trials in each of the tasks.

Due to the large control sample size obtained in this study we were able to standardize the eye movement control scores by age. For all three saccadic eye movement tasks, there was a significant improvement in task performance with increasing age. Therefore, the data were age
corrected by calculating a standard score (t-score) for each age. Scores for the FASD and PAE groups were then calculated using the age-dependent t-score equation obtained from the control group. Differences between groups were analyzed using a one-way ANOVA coupled with Tukey’s Multiple Comparison Test. Effect sizes were also calculated for the dependent variables using Cohen’s \( d \) scores. Data are expressed as mean ± s.e.m. for children in the FASD (n=69; average age 11.6 years, 38 males), control (n=111; average age 10.0 years, 52 males), and PAE (n=13; average age 10.9 years, 6 males) groups.

Saccade measures for all viable trials for the three tasks were assessed by examining the working memory and visuospatial outcome measures. For the prosaccade and antisaccade tasks the error in the saccade endpoint was defined as the angle between the ideal path and the trajectory of the first saccade toward the goal (drawn as a straight line from the beginning to the end of the saccade) (for more details see Paolozza et al., 2013). In the memory-guided task, individual trials were assigned as either correct, timing errors (saccades initiated before 90ms after the go signal), and/or sequence errors (initial saccade made closer to the second peripheral target location than to the first target, therefore in the wrong sequence).

5.3.3 Psychometric Tests:

The NEPSY-II is a standardized psychometric test battery for children 3-16 years of age (Korkman, Kirk, & Kemp 2007). This battery assesses multiple domains of executive functioning, memory, sensorimotor functioning, social perception, language, and visuospatial processing. The Working Memory Test Battery (WMTB) is a standardized psychometric test battery for children 5-15 years of age (Gathercole & Pickering 2001). This battery assesses working memory with subtests measuring central executive, phonological loop and visuospatial sketchpad components of working memory based on Baddeley’s model (Baddeley 2000). Each
subtest used included a teaching example and a practice round where the experimenter had the opportunity to ensure the child understood the instructions and correct the child’s mistakes.

5.3.3.1 Working memory psychometric subtests:

5.3.3.1.1 Animal Sorting (ages 7-16)

This subtest is a card-sorting task that measures concept formation, ability to apply concepts, and set shifting, each of which requires a functioning working memory. The child is shown eight cards and is asked to look for ways that the cards are the same and different. The child must then come up with different, self-initiated ways to sort the cards into two groups of four. An error occurs when the child completes a card sort that is not a correct sort or the child repeats a card sort. The test is discontinued after 360 seconds of cumulative sort time.

5.3.3.1.2 Digit Recall (ages 5-15)

This subtest measures verbal/phonological working memory. The child listens to a string of numbers (1 per second) and attempts to repeat these numbers in the same order. This is presented in a span paradigm where the numbers of digits increase incrementally until the child makes three errors in a row, at which time the task is discontinued. The number and span of correct trials are recorded.

5.3.3.1.3 Block Recall (ages 5-15)

This subtest measures spatial working memory. The child watches the experimenter tap a sequence of blocks in a three-dimensional array and attempts to reproduce that sequence in the same order. This is presented in a span paradigm where the number of blocks is increased incrementally until the child makes three errors in a row, at which time the task is discontinued. The number and span of correct trials are recorded.
5.3.3.2 Visuospatial psychometric subtest:

5.3.3.2.1 Arrows (ages 5-16)

This subtest measures the ability of the participant to assess line orientation. The child is shown an array of arrows around a target and asked to say which arrow(s) points to the center of the target. The number of correct trials is recorded. The test is discontinued after five consecutive scores of 0.

5.3.3.3 Data Analysis of Psychometric Measures

Animal Sorting and Arrows yield raw, scaled, and percentile scores based on age. The mean scaled score is 10 with a standard deviation of 3. Digit Recall and Block Recall also yield raw, standardized, and percentile scores based on age. The mean standard score is 100 with a standard deviation of 10. Similar to the eye movement scores, the raw scores improved with age and once age was removed by using the standard scores, the controls all fluctuated around average (10 or 100, depending on the test) as expected for a random population of typically developing control children. Standard scores of correct trials were used for all analyses except Animal Sorting where the combined scaled score was used because this takes into account both correct and error trials. Differences between groups were analyzed using an ANOVA, coupled with Tukey’s Multiple Comparisons Test. Effect sizes were also calculated for the dependent variables using Cohen’s $d$ scores. Data are expressed as mean ± s.e.m. for children in the FASD (n=72; average age 11.5±3 years, 38 males), control (n=90; average age 10.0±3 years, 44 males) and PAE (n=22; average age 10.9±3 years, 10 males) groups.

IQ was not obtained from any of the participants in this study, since IQ has not been consistently shown to affect performance on psychometric tests (Dennis, Francis, Cirino, Schachar, Barnes, & Fletcher 2009), and matching groups on IQ can result in mismatching on
other important variables (Stigler & Miller 1993). Several studies in children and adolescents with FASD have found that executive functioning deficits remain even after controlling for IQ (Olson, Feldman, Streissguth, Sampson, & Bookstein 1998). The degree to which controlling for IQ alters the association of prenatal alcohol exposure to the outcome depends on how closely the measure overlaps with cognitive aspects IQ (Quattlebaum & O'Connor 2012).

5.3.4 Correlation Analysis

Hypothesis-driven Pearson correlations were used to identify whether psychometric scores were associated with eye movement control measures. The scores from each measure were chosen if the FASD group was significantly different from controls. Bonferroni correction for multiple comparisons was applied to the correlational analyses. Children in the FASD (n=64; average age 11.5 years, 37 males) and control (n=61; average age 10.1 years, 29 males) groups, who completed both psychometric testing and eye movement tasks were matched as closely as possible for age and sex. The PAE group was excluded from the correlational analysis because the number of PAE participants who completed both psychometric testing and eye movement tests was too small to run the group as a separate correlation, and they could not be combined with the FASD group because no deficit was found compared to controls.
5.4 Results

5.4.1 Demographic Variables

To investigate the possible role of demographic variables we performed several analyses on age, comorbidities, SES, group subtypes, and ethnicity (Table 5-1). The one-way ANOVA revealed a significant main effect of age (F(2,205) = 4.0, p=0.019); the FASD group was significantly older than the control group. As previously stated, we controlled for age by performing age corrections on all of the outcome measures. Children with FASD with or without a comorbid disorder of ADHD were compared on all measures presented by first visually examining the data, followed by t-tests between those with or without a comorbid disorder of ADHD. In agreement with a previous study (Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009), no differences were found. Due to the low frequency of other comorbid disorders we were only able to investigate ADHD. We next performed ANOVAs to examine subgroup differences by dividing the FASD group into two subgroups (FAS/pFAS and ARND), which were then compared to each other and to the control and PAE group. No group differences were found between the two FASD groups (FAS/pFAS and ARND) on any psychometric or eye movement measure. Given that the FAS/pFAS group is relatively small compared to our ARND group, differences may be detected between diagnostic subgroups with a larger sample size. The SES was examined for group differences using an ANOVA and the control group was found to have a significantly higher status than both the FASD and PAE group (F(2,173)= 6.7, p=0.0015). However, the SES showed no significant relationship to any of the psychometric or eye movement measures in any group. This was analyzed by both visually examining the data and subsequently running Pearson’s correlations. Ethnicity was also analyzed since the majority of the control group (96%) was Caucasian and the majority of our FASD
group was First Nations (61%). This was accomplished by dividing the FASD group into those who are identified as First Nations and those with any other ethnicity (primarily Caucasian). The data were then examined visually to look for any trending disparities, and then t-tests were run to look for significant differences. There were no significant differences on any of the psychometric and eye movement measures between the two ethnic groupings represented among the FASD participants. Therefore, comorbidities, diagnostic subgroup, SES, and ethnicity do not influence the data and did not need to be included as covariates. There was an imbalance in the ethnicity of the control and FASD groups, and while this does not appear to affect the data it will be important in the future to better match these groups.

5.4.2 Eye Movement Control

Children with FASD performed significantly worse than controls on the prosaccade, antisaccade, and memory-guided saccade tasks (see Table 5-2). The one-way ANOVA revealed a significant effect of group for saccade endpoint error in the prosaccade task (F(2,189) = 6.1, p=0.0028) (Fig. 5-1A); the FASD group was significantly less accurate than the control, but not the PAE group. Similarly, the FASD group displayed significantly less accurate saccade endpoints compared to the control group in the antisaccade task (F(2, 177) = 10.24, p<0.0001) (Fig. 5-1B). Finally, the one-way ANOVA revealed a significant effect of group for sequence errors in the memory-guided task (F(2,162)=6.4, p=0.0022) (Fig. 5-1C); the FASD group made significantly more errors than the control group.
Table 5-2 Mean and effect size (Cohen’s $d$) statistics on cognitive tests.

<table>
<thead>
<tr>
<th>Test</th>
<th>FASD (mean ±SEM)</th>
<th>PAE (mean ±SEM)</th>
<th>Con (mean ±SEM)</th>
<th>Con vs. FASD ($d$)</th>
<th>Con vs. PAE ($d$)</th>
<th>FASD vs. PAE ($d$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Sorting</td>
<td>6.7±0.3</td>
<td>9.6±0.7</td>
<td>9.3±0.4</td>
<td>0.91*</td>
<td>-0.082</td>
<td>-1.05†</td>
</tr>
<tr>
<td>Digit Recall</td>
<td>82.9±1.6</td>
<td>93.8±2.9</td>
<td>100.1±1.8</td>
<td>1.15*</td>
<td>0.38</td>
<td>-0.84†</td>
</tr>
<tr>
<td>Block Recall</td>
<td>87.1±2.0</td>
<td>94.8±2.9</td>
<td>100.5±1.8</td>
<td>0.82*</td>
<td>0.27</td>
<td>-0.65</td>
</tr>
<tr>
<td>Arrows</td>
<td>8.8±0.3</td>
<td>9.2±0.7</td>
<td>10.2±0.3</td>
<td>0.51*</td>
<td>0.20</td>
<td>-0.26</td>
</tr>
<tr>
<td>Pro Endpoint</td>
<td>58.7±24.7</td>
<td>51.2±10.1</td>
<td>49.9±9.5</td>
<td>-0.47*</td>
<td>-0.13</td>
<td>0.40</td>
</tr>
<tr>
<td>Anti Endpoint</td>
<td>65.1±33.3</td>
<td>51.2±14.1</td>
<td>49.8±9.5</td>
<td>-0.63*</td>
<td>-0.11</td>
<td>0.54</td>
</tr>
<tr>
<td>Sequence Errors</td>
<td>58.1±20.5</td>
<td>54.6±10.7</td>
<td>49.6±9.2</td>
<td>-0.86*</td>
<td>-.50</td>
<td>0.21</td>
</tr>
</tbody>
</table>

*p<0.05 compared to controls; †p<0.05 compared to PAE group
Figure 5-1 Saccadic eye movement working memory and visuospatial measures. Data are mean ± s.e.m. for subjects in the control group (n=111) shown in blue, FASD group (n=69) shown in red, and the PAE group (n=13) shown in purple. A: Prosaccade endpoint error. B: Antisaccade endpoint error. C: Memory-guided sequence errors. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared with control subjects.
5.4.3 Psychometric Testing

The FASD group achieved test scores that were significantly lower than controls on all psychometric tests previously mentioned (see Table 5-2). The FASD group was significantly lower than both the control group and the PAE group for scores on Animal Sorting (F(2,159) = 16.9, p<0.0001) (Fig. 5-2A) and Digit Recall (F(2, 175) = 25.8, p<0.0001) (Fig. 5-2B). The one-way ANOVA was significant for the Arrows subtest (F(2,179) = 4.5, p=0.013) (Fig. 5-2C); only the FASD group was significantly lower than the control group. The FASD group also was significantly lower than the control group for scores on Block Recall (F(2,174) = 13.3, p<0.0001) (Fig. 5-2D).
Figure 5-2 Psychometric working memory and visuospatial measures. Data are mean ± s.e.m. for subjects in the control group (n=88) in blue, FASD group (n=71) in red, and the PAE group (n=20) in purple. A: Animal Sorting subtest. B: Digit Recall subtest. C: Block Recall subtest. D: Arrows subtest. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared with control subjects.
5.4.4 Correlations

The results of the psychometric and eye movement correlations can be found in Table 5-3. As hypothesized, there were no significant correlations among the control group for working memory psychometric and eye movement scores (Fig. 5-3), indicating that age was the major factor influencing task performance in this group. In contrast, the FASD group did show a negative correlation between sequence errors in the memory-guided task and Animal Sorting (p= 0.012, r= -0.34), Digit Recall (p=0.013, r= -0.33), and Block Recall (p=0.007, r= -0.35) in the psychometric testing (Fig.5-3). There were also no significant correlations among the control group for visuospatial measures (Fig. 5-4). In contrast, the FASD group showed a negative correlation between the prosaccade saccade endpoint error and performance of the Arrows subtest (p=0.008, r= -0.33), but there was no relationship between antisaccade saccade endpoint error and Arrows performance (Fig.5-4).
Table 5-3 Pearson correlations between psychometric and eye movement tests

<table>
<thead>
<tr>
<th>Correlational Test</th>
<th>FASD p-value</th>
<th>FASD Pearson r</th>
<th>Con p-value</th>
<th>Con Pearson r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Sorting &amp; Sequence Errors</td>
<td>0.012</td>
<td>-0.34</td>
<td>0.23</td>
<td>0.19</td>
</tr>
<tr>
<td>Digit Recall &amp; Sequence Errors</td>
<td>0.013</td>
<td>-0.33</td>
<td>0.33</td>
<td>-0.16</td>
</tr>
<tr>
<td>Block Recall &amp; Sequence Errors</td>
<td>0.0070</td>
<td>-0.35</td>
<td>0.74</td>
<td>-0.053</td>
</tr>
<tr>
<td>Arrows &amp; Prosaccade Endpoint</td>
<td>0.0076</td>
<td>-0.33</td>
<td>0.19</td>
<td>-0.17</td>
</tr>
<tr>
<td>Arrows &amp; Antisaccade Endpoint</td>
<td>0.65</td>
<td>-0.057</td>
<td>0.31</td>
<td>-0.14</td>
</tr>
</tbody>
</table>
Figure 5-3 Correlation of sequence errors from the memory-guided task and working memory subtests from the psychometric tests. Data are individual data points for subjects in the control (n=61) and FASD (n=56) groups. A: Correlation of control group sequence errors and digit recall subtest. B: Correlation of FASD group sequence errors and digit recall subtest. C: Correlation of control group sequence errors and Block Recall subtest. D: Correlation of FASD group sequence errors and Block Recall subtest.
Figure 5-4 Correlation of saccade endpoint errors from the prosaccade and antisaccade tasks and the visuospatial subtest from the psychometric testing. Data are individual data points for subjects in the control (n=61), FASD (n=64) groups. A: Correlation of control group prosaccade endpoint error and Arrows subtest. B: Correlation of FASD group prosaccade endpoint error and Arrows subtest. C: Correlation of control group antisaccade endpoint error and Arrows subtest. D: Correlation of FASD group antisaccade endpoint error and Arrows subtest.
5.5 Discussion

5.5.1 General Findings

The objective of this study was to examine the relationship between measures of working memory and visuospatial skills in children prenatally exposed to alcohol obtained using different assessment tools. Eye movement control tasks have been used extensively to probe sensory, motor and cognitive function across all stages of development, and there is a large body of literature describing the brain structures that contribute to the different aspects of eye movement control. Thus, the performance of eye movement control tasks has been used to assess brain function in a number of clinical conditions such as schizophrenia, Parkinson’s, Huntington’s, stroke, etc. (Ramat, Leigh, Zee, & Optican 2007). In the current study, children with FASD made significantly more sequence errors on the memory-guided saccade task (i.e., failing to recall the order of target presentation), which suggests a deficit in spatial working memory. Similarly, children with FASD performed significantly worse on the psychometric tests that specifically assess working memory (Digit Recall, Block Recall), as well as a test that is at least partially dependent on working memory (Animal Sorting). Within the FASD group, all three psychometric tests were found to be negatively correlated with sequence errors in the memory-guided saccade task. In contrast, there were no significant correlations within the control group among any of these outcome measures because the effect of age, the major contributing factor to task performance, was removed by the use of age-corrected standard scores. That is, in healthy controls the age-corrected performance on the eye movement and psychometric tests of working memory fluctuates around the mean for all ages. Therefore, the performance of these tasks within the control group is quite homogeneous once age is adjusted and as a consequence no correlation was found between these parameters. The fact that we still see a relationship in the FASD group
indicates that something other than age is driving this correlation. We hypothesize that this additional factor is the brain injury induced by prenatal alcohol exposure. We next looked at visuospatial processing. The FASD group performed significantly poorer than controls on Arrows, the psychometric test of visuospatial processing. The FASD group also made greater endpoint errors in the prosaccade and antisaccade tasks, which indicates poorer visuospatial processing. Among the FASD group, only prosaccade endpoint error negatively correlated with Arrows. There were again no significant correlations in the control group. The results in the FASD group indicate that common brain structures are important for regulating working memory and visuospatial processing, and that brain injury induced by prenatal alcohol exposure affects performance on both psychometric and eye movement control measures. Additionally, the results of this study indicate that eye movement control tasks can assess multiple domains of cognitive function simultaneously.

5.5.2 Working memory

Working memory is the limited capacity system allowing for the temporary storage and manipulation of information (Baddeley 2000). The four component model has been theorized to consist of the central executive which is aided by two ‘subsidiary slave systems’ called the phonological loop and the visuospatial sketchpad (Baddeley 2000). The memory-guided saccade task is assumed to test the visuospatial sketchpad as the participants were required to hold visual information about spatial location and the order of the flashed dots simultaneously in memory. Previously we published that sequence errors were not different between controls and children with FASD (Paolozza, Titman, Brien, Munoz, & Reynolds 2013); however this result came from a smaller FASD sample (n=27) and the scores were not standardized for age as we did not have a large enough control group (n=27). The visuospatial sketchpad is also being examined with
Block Recall (Pickering & Gathercole, 2001). Interestingly, the correlation between sequence errors and block recall displayed the strongest relationship of all the working memory tests assessed. This would indicate that these tasks are the most similar and are most likely assessing the same underlying construct of working memory. Digit recall assesses the phonological loop which is assumed to hold verbal and acoustic information (Pickering & Gathercole, 2001). The relationship still holds between this task and sequence errors, indicating a deficit in the central executive as it serves both systems and a deficit in this component would affect working memory overall (Baddeley 2000). This is further supported by Carmichael-Olsen et al. (1998), who found deficits in the central executive in adolescents with FASD.

Animal sorting assesses concept formation, the ability to apply concepts, and set shifting, which require a functioning working memory to successfully complete the task. The participant must simultaneously remember what sorts have already been completed, while also generating new sorts using the provided cards. Similar tasks have been hypothesized to assess abstract working memory (Baddeley 2000). Animal Sorting requires a functioning phonological loop and central executive as well as the episodic buffer (Baddeley 2000). The episodic buffer is a limited-capacity temporary storage system that is capable of integrating information from a variety of sources and serves as an interface between a range of working memory systems. This component is also crucially dependent on executive-based attention (Baddeley 2000). This poor working memory in children can contribute to poor academic performance, hyperactivity, forgetting, and disruptive classroom behaviours (Alloway, Gathercole, Kirkwood, & Elliott 2009).

5.5.3 Visuospatial Processing

Visuospatial skills allow for objects to be perceived and spatial relationships among those objects to be assessed. In agreement with previous research, the saccade endpoint error was
significantly greater in the FASD group for both the prosaccade and antisaccade tasks (Paolozza, Titman, Brien, Munoz, & Reynolds 2013). This deficit may be due to abnormal connections between the cerebellum and other cortical brain regions, as the cerebellum has indirect connections with the cerebral cortex, basal ganglia, and thalamus, and these pathways participate in the online correction of saccade trajectories (Chen-Harris et al. 2008; Glickstein and Doron 2008; Soetedjo et al. 2009). Arrows subtest assesses line orientation judgment, which involves similar brain structures. For example, judgment of line orientation tasks have been found to be impaired in Parkinson’s disease patients, indicating a role of the basal ganglia (Muslimovic et al. 2007). Additionally, cerebellar patients were also observed to have poor line orientation judgment, indicating a role of the cerebellum in this task (Lee et al. 2005). Importantly, children with FASD have been found to have both cerebellar and basal ganglia abnormalities (Mattson, Riley, Sowell, Jernigan, Sobel, & Jones 1996; O’Hare et al. 2005). The relationship between Arrows performance and saccade endpoint error was only present for the prosaccade task. This is because as with Arrows, only the prosaccade task has a visual target to guide the participant’s response. Therefore, this relationship is specific to visually-guided visuospatial processing. Poor visuospatial processing in children with FASD suggests that they may view materials in a disorganized and disconnected manner that inhibits their ability to integrate information and attend to details in a meaningful way.

5.5.4 Linking working memory and visuospatial processing

Visual processing has generally been divided into dorsal and ventral streams. Both working memory and visuospatial processing take place in the dorsal stream. The dorsal stream is thought to give rise to three pathways: parieto-prefrontal, parieto-premotor, and parieto-medial temporal pathways (Kravitz et al. 2011). The parieto-prefrontal pathway mainly involves the
lateral intraparietal, middle temporal, and medial superior temporal areas, which links the occipito-parietal circuit with the caudal portions of the prefrontal cortex (Cavada and Goldman-Rakic 1989; Schall et al. 1995). This pathway is thought to control spatial working memory and would therefore be involved with the memory-guided task and block recall (Kravitz, Saleem, Baker, & Mishkin 2011). Non-human primate research has implicated the prefrontal cortex in working memory (Goldman-Rakic 1995), and structural imaging studies have found abnormalities in the prefrontal cortex in children with FASD (Sowell, Mattson, Kan, Thompson, Riley, & Toga 2008b). Additionally, multiple studies have found that the dorsolateral prefrontal cortex is involved in both task switching and working memory (Goldman and Rosvold 1970; Moore et al. 2009). The parieto-premotor pathway has two parallel projections; one originates in parietooccipital area and the other in the ventral intraparietal area (Gamberini et al. 2009; Rozzi et al. 2006). They both target the premotor cortex and control visually-guided saccades that are necessary to perform the prosaccade and Arrows tasks (Kravitz, Saleem, Baker, & Mishkin 2011). Although working memory and visuospatial processing are distinct they both have at least one overlapping area, the ventral intraparietal area, necessary for the successful completion of the tasks. This may help specify specific brain regions damaged due to prenatal alcohol exposure.

5.5.5 Prenatal Alcohol Exposed Group

Interestingly, the PAE group did not perform worse than the control group on all measures of working memory and visuospatial skills. However, in our previous paper, we found that this same group did perform poorer than controls on measures of attention, switching, and inhibition (Paolozza, Rasmussen, Pei, Hanlon-Dearman, Nikkel, Andrew, McFarlane, Samdup, & Reynolds 2014a). This indicates that in this population working memory and visuospatial
skills may be CNS domains that appear relatively spared. This may, at least partially, explain why this group did not go on to receive a diagnosis. This also points to the importance of common diagnostic tools when assessing those with prenatal alcohol exposure due to the wide range of outcomes displayed by this group (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). This group presents a unique opportunity, not previously presented in the literature, to study psychometric and eye movement measures of working memory and visuospatial skills in a subset of the population who do not have a diagnosis but have been exposed to alcohol prenatally. This also indicates the need for future research to investigate if some CNS functions and patterns of brain damage are more vulnerable to prenatal alcohol exposure than others.
5.6 Conclusions

The results of this study have important clinical and psychological implications. The psychometric tests and eye movement tasks point to specific overlapping brain regions damaged by prenatal alcohol exposure. Due to the extensive research and brain mapping from previous research in eye movement control these tasks can be leveraged to better understand what is being assessed by psychometric testing. Additionally, it also indicates that eye movement tasks may be a viable option as a screening or adjunct tool in the assessment of FASD. The process to receive a diagnosis of an FASD is long, can be stressful on the child and family, requires specialized training, has high costs, and may not be accessible to some rural populations. Therefore, there is potential for eye movement control to be added as a first screening step in this process as the three tasks presented in the study take less than one hour to administer and can simultaneously measure multiple cognitive domains including those presented here, as well as response inhibition (Paolozza, Rasmussen, Pei, Hanlon-Dearman, Nikkel, Andrew, McFarlane, Samdup, & Reynolds 2014a). Using eye movement control tasks as a screening tool can also help with early identification of those in need of a follow-up assessment or those in need of early intervention while waiting for a diagnostic assessment. This could then lead to earlier interventions and potentially less adverse outcomes for those prenatally exposed to alcohol (Streissguth, Bookstein, Barr, Sampson, O’Malley, & Young 2004).
5.7 Acknowledgements

We thank the participants and their families for taking part in the study. This work was supported by NeuroDevNet, which is funded by the Networks of Centres of Excellence, a program of the federal government to advance science and technology. We also acknowledge the NeuroDevNet Neuroinformatics Core for data management system implementation and support and Donald Brein for his technical expertise in the collection and analysis of the eye movement data.
Response inhibition deficits in children with Fetal Alcohol Spectrum Disorder: relationship between diffusion tensor imaging of the corpus callosum and eye movement control

6.1 Abstract

Response inhibition is the ability to suppress irrelevant impulses to enable goal-directed behaviour. The underlying neural mechanisms of inhibition deficits are not clearly understood, but may be related to white matter connectivity, which can be assessed using diffusion tensor imaging (DTI). The goal of this study was to investigate the relationship between response inhibition during the performance of saccadic eye movement tasks and DTI measures of the corpus callosum in children with or without Fetal Alcohol Spectrum Disorder (FASD). Participants included 43 children with an FASD diagnosis (12.3 ± 3.1 years old) and 35 typically developing children (12.5 ± 3.0 years old) both aged 7-18, assessed at three sites across Canada. Response inhibition was measured by direction errors in an antisaccade task and timing errors in a delayed memory-guided saccade task. Manual deterministic tractography was used to delineate six regions of the corpus callosum and calculate fractional anisotropy (FA), mean diffusivity (MD), parallel diffusivity, and perpendicular diffusivity. Group differences in saccade measures were assessed using t-tests, followed by partial correlations between eye movement inhibition scores and corpus callosum FA and MD, controlling for age. Children with FASD made more saccade direction errors and more timing errors, which indicates a deficit in response inhibition. The only group difference in DTI metrics was significantly higher MD of the splenium in FASD compared to controls. Notably, direction errors in the antisaccade task were correlated negatively to FA and positively to MD of the splenium in the control, but not the FASD group, which
suggests that alterations in connectivity between the two hemispheres of the brain may contribute to inhibition deficits in children with FASD.
6.2 Introduction

Response inhibition is the ability to suppress irrelevant stimuli or behavioural impulses to enable goal-directed behaviour. Evidence from functional neuroimaging, animal models and human lesion studies indicate that the prefrontal cortex (Aron & Poldrack 2005; Iversen & Mishkin 1970), anterior cingulate cortex (Liddle et al. 2001), and corpus callosum (Bearden et al. 2011; Gadea et al. 2009; Stewart et al. 2003) all play a vital role in response inhibition. Eye movement control tasks have been used to measure response inhibition in typically developing children across a wide age range and have found that inhibitory skill increases with age (Hwang, Velanova, & Luna 2010). Saccades are rapid eye movements that bring new visual targets onto the fovea of the retina and require multiple brain regions for successful execution. Several brain regions such as the dorsolateral prefrontal cortex (DeSouza, Menon, & Everling 2003; Funahashi, Chafee, & Goldman-Rakic 1993), lateral intraparietal area (Gottlieb & Goldberg 1999; Schlag-Rey, Amador, Sanchez, & Schlag 1997; Zhang & Barash 2000), frontal eye fields (Everling & Munoz 2000), secondary eye fields (Amador, Schlag-Rey, & Schlag 2004; Schlag-Rey, Amador, Sanchez, & Schlag 1997), basal ganglia (Clark et al. 2000; Lebel et al. 2011), and superior colliculus (Everling, Dorris, & Munoz 1998a; Everling, Dorris, Klein, & Munoz 1999) have been associated with eye movement control. However, less is known about the role that white matter tracts play in eye movement control. Successful saccades most likely involve the corpus callosum, the largest white matter structure in the brain, which links homologous areas in the right and left hemispheres. This study investigates the corpus callosum in terms of its relationship with eye movement control (Bruni and Montemurro 2009). More specifically, the splenium, the most posterior sector of the corpus callosum, has been linked to the striate and extrastriate visual areas which are cortical targets implicated in sensorimotor processing (Putnam
Eye movement control tasks have emerged as a portable and cost-effective method which can effectively measure cognitive, sensory, and motor functions in different clinical populations (Ramat, Leigh, Zee, & Optican 2007). For example, eye movement control measures have been used to characterize deficits in executive functions and motor control in children who have prenatal alcohol exposure (Green, Munoz, Nikkel, & Reynolds 2007; Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009; Paolozza, Titman, Brien, Munoz, & Reynolds 2013). Prenatal alcohol exposure induces a spectrum of adverse effects that can be categorized into several diagnostic subgroups, collectively referred to as Fetal Alcohol Spectrum Disorder (FASD) (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). Eye movement tasks have found that error rates in the antisaccade task, which requires participants to suppress an automatic response towards a target and instead make a voluntary saccade in the opposite direction, are significantly elevated in children with FASD (Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009; Paolozza, Titman, Brien, Munoz, & Reynolds 2013). Additionally, in a previous study that utilized a memory-guided saccade task which requires the participant to remember the spatial location and sequence of presentation of two visual targets (Paolozza, Titman, Brien, Munoz, & Reynolds 2013), we showed that children with FASD were unable to inhibit the automatic response and looked to the visual targets before receiving the appropriate go signal. These studies show that the suppression of automatic saccades coupled with the generation of voluntary saccades by a goal-directed plan is adversely affected in FASD.

Previous structural magnetic resonance imaging (MRI) and autopsy studies have reported widespread brain injury in those diagnosed with FASD in many of the aforementioned cortical
gray matter regions and white matter including the corpus callosum (Autti-Ramo, Autti, Korkman, Kettunen, Salonen, & Valanne 2002;Clarren and Smith 1978;Riley, Mattson, Sowell, Jernigan, Sobel, & Jones 1995;Swayze, Johnson, Hanson, Piven, Sato, Giedd, Mosnik, & Andreasen 1997). Diffusion tensor imaging (DTI) can examine white matter integrity by measuring water diffusion in the brain. This method allows for the reconstruction of individual white matter pathways and provides quantitative measures, such as fractional anisotropy (FA) and mean diffusivity (MD), presumed to reflect cellular properties such as myelination and coherence/packing of axons (Beaulieu 2002). DTI studies of the corpus callosum in FASD populations have revealed abnormalities of FA and MD in various tracts (Fryer, Schweinsburg, Bjorkquist, Frank, Mattson, Spadoni, & Riley 2009;Lebel, Rasmussen, Wyper, Walker, Andrew, Yager, & Beaulieu 2008;Lebel, Rasmussen, Wyper, Andrew, & Beaulieu 2010;Li, Coles, Lynch, & Hu 2009;Ma, Coles, Lynch, Laconte, Zurkiya, Wang, & Hu 2005;Sowell et al. 2008a;Wozniak, Mueller, Chang, Muetzel, Caros, & Lim 2006;Wozniak, Muetzel, Mueller, McGee, Freerks, Ward, Nelson, Chang, & Lim 2009). FA measures in the corpus callosum have correlated with saccadic reaction time in two eye movement tasks in children with FASD (Green, Lebel, Rasmussen, Beaulieu, & Reynolds 2013); however, this paper had no control group and a relatively small sample size (n=14). Additionally, it used a voxel-based analysis of the corpus callosum, which relies heavily on adequate spatial normalization that can be problematic for the corpus callosum (Snook et al. 2007). Tractography of individual white matter tracts overcomes this limitation and yields diffusion parameters averaged over an entire tract rather than individual voxels.

This paper reports the findings from the first DTI tractography study to examine white matter integrity in relation to performance on inhibition measures obtained from two eye
movement control tasks, including a memory-guided saccade task, in a cohort of children with FASD recruited in the multi-site NeuroDevNet study (Reynolds, Weinberg, Clarren, Beaulieu, Rasmussen, Kobor, Dube, & Goldowitz 2011). We hypothesized that FA and MD of the corpus callosum, as well as saccadic eye movements will be significantly different in children with FASD (n=43, age range 7-18 years) when compared to controls (n=35, age range 7-18 years). We also hypothesized that these measures will be related to one another.
6.3 Methods

6.3.1 Participants

Participants aged 7-18 years were recruited at three sites across Canada and had either a confirmed diagnosis of FASD (n=47) or were typically developing control children (n=41). Children with FASD were previously assessed and diagnosed according to the Canadian Guidelines for FASD Diagnosis (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005) and were recruited through diagnostic clinics in Kingston, ON, Ottawa, ON, Edmonton, AB, Cold Lake, AB, and Winnipeg, MB, as part of a larger study funded by NeuroDevNet (Reynolds, Weinberg, Clarren, Beaulieu, Rasmussen, Kobor, Dube, & Goldowitz 2011). The performance on a battery of psychometric tests and the link with eye movement control has been previously reported in a larger cohort from which the current subsample with both DTI and eye tracking was extracted (Paolozza, Rasmussen, Pei, Hanlon-Dearman, Nikkel, Andrew, McFarlane, Samdup, & Reynolds 2014a;Paolozza, Rasmussen, Pei, Hanlon-Dearman, Nikkel, Andrew, McFarlane, Samdup, & Reynolds 2014b). All experimental procedures were reviewed and approved by the Human Research Ethics Boards at Queen’s University, University of Alberta, Children’s Hospital of Eastern Ontario, and the University of Manitoba. Written informed consent was obtained from a parent or legal guardian and assent was obtained from each child before study participation. Due to quality control measures (movement, braces, etc), four participants with FASD were excluded from analysis, leaving 43 FASD participants with adequate DTI data, scanned in either Kingston (n=18; mean age=12.6±3.4; 9 males), Edmonton (n=15; mean age=11.5±3.3; 8 males), or Winnipeg (n=10; mean age=12.9±1.5; 5 males). Typically developing control children (n=41) were recruited from the same geographical areas and six were excluded due to either a pre-existing disorder or failing quality control measures, leaving
35 control participants scanned in Kingston (n=14; mean age=13.8±3.1; 8 males), Edmonton (n=12; mean age=11.6±2.7; 3 males), or Winnipeg (n=9; mean age=11.1±2.7; 2 males).

Participant information is summarized in Table 6-1. Socioeconomic status (SES) was calculated using Hollingshead’s Four-Factor Index of Social Status for the FASD and control groups and analyzed for group differences (Hollingshead 1975). Study data were collected and managed using REDCap electronic data capture tools (Harris, Taylor, Thielke, Payne, Gonzalez, & Conde 2009) and LORIS imaging database hosted at Queen’s University (Das et al. 2011).
Table 6-1 Demographic characteristics

<table>
<thead>
<tr>
<th>Diagnostic Subtype n (%)</th>
<th>Control (n=35)</th>
<th>FASD (n=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAS</td>
<td>-</td>
<td>3 (7)</td>
</tr>
<tr>
<td>pFAS</td>
<td>-</td>
<td>9 (21)</td>
</tr>
<tr>
<td>ARND</td>
<td>-</td>
<td>31 (72)</td>
</tr>
</tbody>
</table>

Demographics: | t-test p-value |
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</thead>
<tbody>
<tr>
<td>Mean age±SD (range)</td>
<td>12.5±3.0 (7-18)</td>
</tr>
<tr>
<td>Males n (%)</td>
<td>14 (39)</td>
</tr>
<tr>
<td>Right handed n (%)</td>
<td>35 (97)</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>47</td>
</tr>
</tbody>
</table>

Ethnicity: | Chi-squared p-value |
<table>
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<th></th>
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</tr>
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<tbody>
<tr>
<td>Caucasian n (%)</td>
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<tr>
<td>First Nations n (%)</td>
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<td>Other Ethnicity n (%)</td>
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</tbody>
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Comorbidities n (%): | Chi-squared p-value |
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<td>ODD</td>
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<td>Anxiety</td>
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<td>Depression</td>
<td>0 (0)</td>
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<tr>
<td>Other</td>
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</table>

Medications n (%): | Chi-squared p-value |
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<thead>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulants</td>
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<tr>
<td>Antipsychotics</td>
<td>0 (0)</td>
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<tr>
<td>Antidepressant</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
### 6.3.2 Saccadic eye movement recordings

Participants were seated comfortably in a dark, quiet room on a stable chair and instructions for each trial were given verbally, and repeated before each task started. Eye position was recorded using the Eyelink 1000 (SR Research, Kanata, ON), using previously described methods (Paolozza, Rasmussen, Pei, Hanlon-Dearman, Nikkel, Andrew, McFarlane, Samdup, & Reynolds 2014a). In the antisaccade task, each trial started with illumination of a central fixation point (FP), which then disappeared and, after a delay of 200 ms (gap period), a peripheral target appeared randomly at 10° to the left or right of the central FP. Participants were instructed to complete a saccade in the opposite direction of the target. No error feedback was given. One block of 60 trials was obtained from each participant. In the memory-guided saccade task, participants were instructed to maintain fixation on the central FP, after which two peripheral targets appeared in immediate succession and participants were required to fixate on the central FP for an additional 0, 600, 1200, or 1800 ms (randomly allocated) between the disappearance of the second peripheral target and the disappearance of the FP. After the FP and peripheral targets disappeared, participants were required to make two saccades as accurately as possible to these locations in the same sequence. A single block of 72 trials was collected for this task.

### 6.3.3 Quality Control of Eye Tracking Measures

Data were analyzed using custom software developed in MATLAB (R2009b, The Mathworks, Inc, Natick, Massachusetts). Saccades were defined as having a speed of greater than 2.5 times the standard deviation of the background noise (measured during fixation) for at least 5 continuous sample points in time. The only trials used were those for which the participant was fixating on the FP at the appropriate time. If the participant broke fixation inappropriately (i.e. not to a target location or away from the screen) the trial was discarded from
analysis. Any trials where eye tracking was lost were removed in the analysis, and to be included each participant had to achieve greater than 50% viable trials in each of the tasks.

6.3.4 Saccade Outcome Measures

Saccade performance for all viable trials for the two tasks was assessed by examining several outcome measures. Saccadic reaction time (SRT) in the antisaccade task were defined as the time from the appearance of the peripheral target to the initiation of the first saccade during a correct trial. Direction errors in the antisaccade task were defined as any initial saccade in the wrong direction with respect to the instruction (i.e., towards the target). Anticipatory errors were defined as any saccade to one of the two target locations before the appearance of the target itself can be perceived (i.e. within 90 ms after peripheral target appearance).

In the memory-guided task, SRT of both the first and second saccades were calculated from disappearance of the central FP during a correct trial. Individual trials were assigned as either correct, timing errors (saccades initiated before 90 ms after the go signal), and/or sequence errors (initial saccade made closer to the second peripheral target location than to the first target, therefore in the wrong sequence).

Eye movement measures were age-corrected using the entire NeuroDevNet control cohort (n=102, mean age 10.4 years) to calculate a standardized t-score equation for each age. Standard scores for the control and FASD groups in the current study were then calculated using the age-dependent t-score equation obtained from the larger control group. Differences between groups were analyzed using a t-test.

6.3.5 Image Acquisition

Brain MRI was collected at three sites (Edmonton, AB, 1.5T Siemens Sonata; Kingston, ON, and Winnipeg, MB, both 3T Siemens Trio), including DTI, T1-weighted (not used here),
T2-weighted (not used here), fluid-attenuated inversion recovery (FLAIR; not used here), and resting state functional MRI (not used here) scans for a total scan time of about 20 mins. All DTI was acquired using a dual spin-echo echo planar imaging sequence with: 2.2 mm isotropic voxels, 50 axial-oblique slices with no interslice gap; 30 non-collinear diffusion sensitizing gradient directions with b= 1000 s/mm$^2$; 5 (Edmonton) or 1 (Kingston and Winnipeg) b= 0 s/mm$^2$; 1 average; FOV= 220 x 220 mm$^2$; matrix of 96 x 96; TE= 94 ms; TR= 6600 ms (Kingston and Winnipeg) or TR= 7700 ms (Edmonton); acquisition time 3.46 minutes.

6.3.6 Tractography

Manual deterministic tractography of the corpus callosum was performed in ExploreDTI (Leemans et al., 2009) by a single operator (AEP), blinded to participant group, age, sex, and handedness. The corpus callosum was divided into six segments (genu, rostral body, anterior midbody, posterior midbody, isthmus, and splenium) according to a previously defined method (Witelson, 1989). Seed regions of interest (ROI) were manually placed on the same mid-sagittal slice and “Not” ROIs were used to exclude any spurious tracts (Figure 6-1). FA, MD, parallel diffusivity ($\lambda_{II}$), and perpendicular diffusivity ($\lambda_{I}$) were calculated (averaging across all voxels in a given tract) and compared between the two groups using a one-way ANCOVA, with age as a covariate. Test-retest reliability was determined by measuring FA of each tract twice each on a random subset of Kingston participants (n=15). Intraclass correlation coefficient of test-retest FA values within individuals yielded a high reliability (r=0.922-0.957).
Figure 6-1 Tractography of the corpus callosum. The corpus callosum was manually seeded into six inter-hemispheric tracts (anterior to posterior). Tract 1 is the genu, tract 2 is the rostral body, tract 3 is the anterior midbody, tract 4 is the posterior midbody, tract 5 is the isthmus, and tract 6 is the splenium.
6.3.7 Reliability Study

Since all three sites had slightly different protocols and scanners, a separate reliability study was performed to determine if data could be combined across sites. Eight healthy adult controls (mean age 28 ± 6 years, 2 males) were scanned twice at each site (getting out of scanner between the two scans), each within a 2 week period (6 scans per person, 48 scans total). The data were then analyzed by the same operator (AEP) in the exact same manner as the study data by dividing the corpus callosum into the same six sub-regions. To examine the operator reliability and scanner consistency, intraclass correlation coefficients (ICC) were run between the two scans at each site for FA (our primary DTI variable) and were found to range from 0.957-0.995, indicating high reliability of the operator and scanner. To analyze the difference between sites, within each participant, the coefficient of variation and ICC were calculated for each participant across all sites for all six tracts. The average coefficient of variation (COV) was 2.02% and ICCs ranged from 0.49 (splenium) to 0.93 (genu) (Figure 6-2). To analyze the difference between participants within sites, the COV and the ICC were calculated for each site using all six scans per subject for all 6 tracts. The mean COV was found to be 3.48% and the ICC ranged from 0.23 (posterior midbody) to 0.73 (genu). We ran the same analysis for MD and found similar results to FA where the mean COV between sites was 3.8% with an ICC ranging from 0.99 (genu) to 0.71 (isthmus), and the mean COV between participants was 3.2% with an ICC ranging from 0.38 (splenium) to 0.96 (genu), indicating that the site introduced less variation in MD than the participants themselves. Given the low variability between scanners, the data for all three sites were combined for the analysis. Additionally, there were no systematic effects where FA was higher at one site than other sites for the eight individuals.
Figure 6-2 Intra- and inter-subject variability between sites. The mean FA value of the two scans at each site are shown for eight participants scanned in Edmonton (Siemens Sonata 1.5T) and Kingston and Winnipeg (both with Siemens Trio 3T). In general, the ranking of the participants with the highest/lowest FA was maintained across all three scanner sites. Thus, the FA for each participant was quite consistent across the three sites with coefficient of variation ranging from 0.4-4.8% (mean 2.0%) for the six tracts (shown at the right of each plot). The site FA variation per individual (shown at the bottom of each plot) was less than the FA variability between the eight participants at each site which ranged from 2.4-5.7% (mean 3.5%).
6.3.8 Correlation Analysis

Hypothesis-driven partial correlations (correcting for age) were used to identify whether the inhibition measures from the eye tracking tasks were associated with FA or MD of each of the six corpus callosum sub-regions, with Holm-Sidak correction for multiple comparisons. Specifically, correlations of direction errors from the antisaccade task and timing errors from the memory-guided task were assessed versus FA and MD of all six corpus callosum tracts. Hypothesis-driven correlations were chosen based on the group differences observed on both the eye movement and DTI scores.
6.4 Results

6.4.1 Eye Movement Control

The FASD group displayed multiple deficits in eye movement control on both the antisaccade and memory-guided tasks (Table 6-2). On the antisaccade task, children with FASD had significantly slower SRT compared to controls, more anticipatory errors, and more direction errors. On the memory-guided saccade task, the FASD group was not different from controls on SRT, but had significantly more sequence errors and timing errors than controls.
Table 6-2 Eye Tracking Measures.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>FASD</th>
<th>p-value</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SEM</td>
<td>range</td>
<td>mean±SEM</td>
<td>range</td>
</tr>
<tr>
<td><strong>Antisaccade Task:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRT (t-score)</td>
<td>50.0±1.8</td>
<td>34.1-73.3</td>
<td>56.4±2.0</td>
<td>30.3-92.7</td>
</tr>
<tr>
<td>Anticipatory (t-score)</td>
<td>50.0±1.7</td>
<td>38.1-76.6</td>
<td>58.5±2.9</td>
<td>23.2-118.2</td>
</tr>
<tr>
<td>Direction errors (t-score)</td>
<td>48.4±1.4</td>
<td>34.5-64.4</td>
<td>55.7±2.1</td>
<td>33.0-102.9</td>
</tr>
<tr>
<td><strong>Memory-guided Task:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SRT 1\textsuperscript{st} saccade (t-score)</td>
<td>49.9±1.5</td>
<td>38.1-72.2</td>
<td>54.8±3.5</td>
<td>26.3-95.0</td>
</tr>
<tr>
<td>SRT 2\textsuperscript{nd} saccade (t-score)</td>
<td>49.3±1.5</td>
<td>31.4-66.8</td>
<td>48.2±1.9</td>
<td>17.1-73.9</td>
</tr>
<tr>
<td>Sequence errors (t-score)</td>
<td>48.8±1.4</td>
<td>36.8-62.7</td>
<td>56.3±3.0</td>
<td>19.6-121.7</td>
</tr>
<tr>
<td>Timing errors (t-score)</td>
<td>49.2±1.7</td>
<td>35.9-71.9</td>
<td>61.4±3.6</td>
<td>37.4-133.0</td>
</tr>
</tbody>
</table>

*indicates significance at p<0.05
6.4.2 DTI Measures

Group differences of either MD or FA between control and FASD were only found for MD of the splenium which was significantly higher in the FASD group compared to the healthy controls, after correcting for age (F(1,79)=11.3, p=0.018; Table 6-3). In the splenium, FA was found to increase with increasing age for both the control (p=0.019) and FASD (p<0.0001) groups (Figure 6-3A & B). Additionally, MD in the splenium was found to decrease with increasing age in both the FASD (p=0.008) and control (p=0.016) groups (Figure 6-3C & D). In the splenium, parallel diffusivity was significantly higher in the FASD group when compared to controls (control mean=1.47±0.06x10^{-3} mm^2/s, FASD mean=1.52±0.07x10^{-3} mm^2/s, F(1,79)=3.3, p=0.004), whereas perpendicular diffusivity was not different (control mean=0.49±0.03x10^{-3} mm^2/s, FASD mean=0.51±0.05x10^{-3} mm^2/s, p=0.15).
Table 6-3 DTI Group Differences.

<table>
<thead>
<tr>
<th>Site</th>
<th>Measure</th>
<th>Control (mean±SD)</th>
<th>FASD (mean±SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genu</td>
<td>FA</td>
<td>0.56±0.02</td>
<td>0.55±0.03</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>MD (mm$^2$/s x 10$^{-3}$)</td>
<td>0.80±0.03</td>
<td>0.81±0.04</td>
<td>0.22</td>
</tr>
<tr>
<td>Rostral Body</td>
<td>FA</td>
<td>0.54±0.02</td>
<td>0.54±0.03</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>MD (mm$^2$/s x 10$^{-3}$)</td>
<td>0.81±0.03</td>
<td>0.82±0.05</td>
<td>0.33</td>
</tr>
<tr>
<td>Anterior Midbody</td>
<td>FA</td>
<td>0.55±0.02</td>
<td>0.55±0.03</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>MD (mm$^2$/s x 10$^{-3}$)</td>
<td>0.81±0.04</td>
<td>0.83±0.04</td>
<td>0.075</td>
</tr>
<tr>
<td>Posterior Midbody</td>
<td>FA</td>
<td>0.55±0.02</td>
<td>0.54±0.04</td>
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<td></td>
<td>MD (mm$^2$/s x 10$^{-3}$)</td>
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<tr>
<td>Isthmus</td>
<td>FA</td>
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<tr>
<td></td>
<td>MD (mm$^2$/s x 10$^{-3}$)</td>
<td>0.83±0.03</td>
<td>0.84±0.04</td>
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<tr>
<td>Splenium</td>
<td>FA</td>
<td>0.60±0.03</td>
<td>0.60±0.03</td>
<td>0.89</td>
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<td></td>
<td>MD (mm$^2$/s x 10$^{-3}$)</td>
<td>0.82±0.03</td>
<td>0.84±0.05</td>
<td>0.018*</td>
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</table>

*indicates significance at p<0.05
Figure 6-3 FA and MD changes with age in the splenium. The correlation between the FA and age is shown for the control (A; n=35) and FASD (B; n=43) groups separately. Both groups show increases in FA as age increases. The correlation between the MD and age is shown for the control (C; n=35) and FASD (D; n=43) groups separately. Both groups show decreases in MD as age increases.
6.4.3 Correlational Analyses

After correcting for multiple comparisons, two significant correlations were found (Table 6-4). The first was a negative correlation between direction errors in the antisaccade task and FA of the splenium within the control group (p=0.001) (Figure 6-4A). This indicates that control participants with higher FA also made fewer errors on the antisaccade task. A positive correlation between the MD of the splenium and direction errors was also significant in the control group (p=0.009) (Figure 6-4C). In contrast, the FASD group showed no significant correlation between FA of the splenium and direction errors in the antisaccade task (p=0.31; Figure 6-4B) or MD of the splenium and direction errors (p=0.87; Figure 6-4D).
Table 6-4 Correlations between FA and MD in the corpus callosum with direction errors from the antisaccade task

<table>
<thead>
<tr>
<th>Antisaccade vs Direction Errors</th>
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<th>FASD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson r</td>
<td>p-value</td>
</tr>
<tr>
<td>Genu FA</td>
<td>-0.19</td>
<td>0.280</td>
</tr>
<tr>
<td>Genu MD</td>
<td>0.30</td>
<td>0.078</td>
</tr>
<tr>
<td>Rostral body FA</td>
<td>-0.11</td>
<td>0.542</td>
</tr>
<tr>
<td>Rostral body MD</td>
<td>0.01</td>
<td>0.937</td>
</tr>
<tr>
<td>Anterior midbody FA</td>
<td>-0.21</td>
<td>0.237</td>
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<tr>
<td>Anterior midbody MD</td>
<td>0.07</td>
<td>0.720</td>
</tr>
<tr>
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<td>-0.09</td>
<td>0.605</td>
</tr>
<tr>
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<td>0.270</td>
</tr>
<tr>
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</tr>
<tr>
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<td>0.11</td>
<td>0.554</td>
</tr>
<tr>
<td>Splenium FA</td>
<td>-0.52</td>
<td>0.001*</td>
</tr>
<tr>
<td>Splenium MD</td>
<td>0.44</td>
<td>0.009*</td>
</tr>
</tbody>
</table>

*indicates significance at p<0.009 (Holm-Sidak multiple comparison correction)
Figure 6-4 DTI-Eye Movement Correlations. The age-corrected splenium FA residuals were negatively correlated to direction error t-score (age corrected score) in the control group (A; n=35) but not the FASD group (B; n=43). Additionally, the age corrected MD residuals were positively correlated to direction errors t-score in the control (C; n=35) but not the FASD (D; n=43) group. Males are shown as circles and females are shown as triangles for both groups.
6.5 Discussion:

6.5.1 General Findings

The current study found group differences between children with FASD and typically developing children in both eye movement control and DTI measures. The children with FASD performed significantly worse on eye movement behavioural measures indicating poor response inhibition, reaction time, and spatial working memory. Group differences were also found in the splenium of the corpus callosum with the FASD group displaying significantly higher MD. This result was primarily driven by a difference in parallel diffusivity, which has been associated with neurotoxicity. For example, administration of the neurotoxin methylmercury to the rat results in an increased parallel but not perpendicular diffusivity, and this was found to be due to reductions in microtubules and neurofilaments in the axoplasm (Kinoshita et al. 1999). Alcohol may also have similar neurotoxic effects as a higher parallel diffusivity has been reported in the anterior thalamic radiation of males with high alcohol use (Hill et al. 2013). There were significant correlations between direction errors in the antisaccade task and FA (negative) or MD (positive) of the splenium of the control group, but not the FASD group. These findings point to alterations in connectivity between the two hemispheres of the brain that may contribute to inhibition deficits in children with FASD.

6.5.2 Eye Movement Control

The current study found that children with FASD had significant difficulties with eye movement control. Behavioural deficits on the antisaccade task included increased number of anticipatory saccades, direction errors and slower SRT. While we have previously reported increased direction errors and slower SRT (Green, Munoz, Nikkel, & Reynolds 2007;Green, Mihic, Brien, Armstrong, Nikkel, Stade, Rasmussen, Munoz, & Reynolds 2009;Paolozza,
In the current study, we also found a significant increase in anticipatory saccades in children with FASD. An increase in anticipatory saccades suggests a problem with top-down control by the prefrontal cortex on cortical and subcortical regions (Clementz et al. 2010), as the children in the FASD group had difficulty waiting for the proper go signal to make a saccade.

Similar to the antisaccade task, the children with FASD displayed increased behavioural errors, specifically more sequence and timing errors, on the memory-guided task. An increase in the frequency of timing errors has consistently been observed in children with FASD compared with controls (Paolozza, Titman, Brien, Munoz, & Reynolds 2013; Paolozza, Rasmussen, Pei, Hanlon-Dearman, Nikkel, Andrew, McFarlane, Samdup, & Reynolds 2014a). In contrast, we did not observe a difference in sequence errors in a smaller sample (n=27) of children with FASD (Paolozza et al., 2013); however, with a larger sample size that enabled standardization for age the more subtle difference in sequence errors has become evident (data of the current study and see also Paolozza et al., 2014b). It is important to fully characterize eye movement performance in children with neurodevelopmental disorders (e.g., errors and metrics), as this may help to establish whether there are (i) unique profiles of deficits that occur in specific disorders and (ii) correlations with certain types of structural or functional brain injury.

### 6.5.3 Diffusion Parameters

The corpus callosum is the largest white matter tract in the brain, connecting homologous regions of the two hemispheres. In this study we segmented the corpus callosum into six distinct regions. The only region that was significantly different between the FASD and control groups was the splenium, the most posterior portion, which connects bilateral aspects of the occipital lobes. Structural MRI studies have suggested that the splenium is the most severely affected
callosal area in those with FASD compared to controls (Bookstein et al. 2007; Sowell et al. 2001; Yang et al. 2012). Several DTI studies have found group differences of lower FA in the splenium (Lebel, Rasmussen, Wyper, Walker, Andrew, Yager, & Beaulieu 2008; Li, Coles, Lynch, & Hu 2009; Sowell, Johnson, Kan, Lu, Van Horn, Toga, O'Connor, & Bookheimer 2008a; Wozniak, Muetzel, Mueller, McGee, Freerks, Ward, Nelson, Chang, & Lim 2009) and higher MD in the splenium of young adults with FASD (Ma, Coles, Lynch, Laconte, Zurkiya, Wang, & Hu 2005); this latter finding is similar to the current study in a group of children and adolescents. These DTI findings suggest that prenatal alcohol exposure may be associated with microstructural differences, e.g. axonal degradation, in the splenium of the corpus callosum.

6.5.4 DTI-Eye Tracking Correlation Analysis

DTI has been used to demonstrate links between corpus callosum microstructural white matter integrity and cognitive functioning in healthy development (i.e. co-ordination and bimanual tasks) (Johansen-Berg et al. 2007; Muetzel et al. 2008), as well as neurodevelopmental disorders (Alexander et al. 2007; Cheng et al. 2013; Fryer, Schweinsburg, Bjorkquist, Frank, Mattson, Spadoni, & Riley 2009; Sundaram et al. 2008). Using tractography, we demonstrate a negative correlation in the control group between FA of the splenium and direction errors in the antisaccade task, as well as a positive correlation between MD of the splenium and direction errors in the antisaccade task. Poorer response inhibition has been correlated with decreased splenium size in adults with bipolar disorder who also display inhibitory control problems (Bearden, van Erp, Dutton, Boyle, Madsen, Luders, Kieseppa, Tuulio-Henriksson, Huttunen, Partonen, Kaprio, Lonnqvist, Thompson, & Cannon 2011). Response inhibition during a dichotic listening task has also been correlated to the area of the posterior corpus callosum in patients with Multiple Sclerosis (Gadea, Marti-Bonmati, Arana, Espert, Salvador, & Casanova 2009).
Finally, in children exposed to polychlorinated biphenyls, the splenium volume has been correlated with psychometric response inhibition, where smaller size was related to greater number of inhibitory errors (Stewart, Fitzgerald, Reihman, Gump, Lonky, Darvill, Pagano, & Hauser 2003). The relationship seen in this study supports the notion that alterations in white matter integrity of the splenium could lead to response inhibition deficits commonly seen in children with FASD. The splenium has been linked to the integration of visual information with the proper motor response in the dorsal/parietal visual pathway (Cabeza and Nyberg 2000;DeYoe et al. 1994), and therefore, direction errors may also be due to an early breakdown of visual information coming into the brain that is not being transmitted to the correct areas of the frontal lobes via indirect connections with the splenium which is required for proper inhibitory control.

We did not find any relationship between DTI measures and timing errors on the memory-guided task possibly due to the complex nature of the task itself. The antisaccade task measures response inhibition directly and only requires the participant to follow one instruction (look to the opposite side of the screen). In contrast, the memory-guided task requires the participant to simultaneously remember several instructions and participants must use previously presented sensory information to plan and initiate a motor response at the appropriate time. This task, therefore, requires the integration of multiple domains of cognitive function, including spatial working memory and response suppression, and would presumably therefore depend on multiple pathways in the brain and not the corpus callosum.

6.5.5 Limitations

There are certain limitations of this study that must be acknowledged. First, the tensor model and deterministic tractography can be prone to error in areas of crossing fibers and may
therefore lead to artificially low FA in such locations, and subsequently do not identify the lateral projections of the corpus callosum. Second, this study only presents data from the corpus callosum and does not include any other tracts in the brain. Therefore, we can point to the splenium as playing a role in inhibition but are unable to determine the potential contribution of other white matter tracts. Third, this tractography analysis used manual seeding ROIs which can have increased variability due to human error. However, all were placed by the same investigator who was blinded to the individual participant’s demographics and performance. Finally, there was an imbalance in the ethnicity of the control and FASD groups, and while this does not appear to affect the data it will be important in the future to better match these groups.
6.6 Conclusions

In the present study, we demonstrate group differences between children with FASD and control participants across many measures of eye movement control. Eye movement tasks hold promise as a future screening tool given that they are efficient, portable and easy to administer. Group differences in MD of the splenium of the corpus callosum indicate that prenatal alcohol exposure may be associated with white matter abnormalities in this area. Response inhibition measures on the antisaccade task in healthy control children correlated negatively with FA and positively with MD of the splenium, which is consistent with the view that interhemispheric connectivity is required to properly integrate information in order to successfully and efficiently inhibit an automatic response and to generate a voluntary response (Madsen et al. 2010). This relationship was not present in children with FASD, who also exhibited evidence of decreased microstructural integrity of the splenium. Future studies should include functional MRI experiments to determine the pattern of brain activation in children with FASD compared with controls during the performance of saccadic eye movement tasks.
6.7 Acknowledgements

We thank the participants and their families for taking part in the study. This work was supported by NeuroDevNet, which is funded by the Networks of Centres of Excellence, a program of the federal government to advance science and technology. We also acknowledge the NeuroDevNet NeuroInformatics Core for data management system implementation and support and Donald Brien for his technical expertise in the collection and analysis of the eye movement data. Alberta Innovates Health Solutions is acknowledged for a salary award (CB) and PhD scholarship (ST).
Chapter 7

General Discussion

7.1 Overall Findings

The overarching goal of this thesis research was to assess the utility of eye movement control tasks, MRI, and behavioural tests as tools that can differentiate children with FASD from typically developing control children. These three techniques have all been used to assess brain function in children, and therefore it was hypothesized that diffusion tensor imaging (DTI), eye tracking and psychometric testing would successfully differentiate children with FASD from control children. Moreover, it was also hypothesized that individual measures of brain structure and function obtained with these techniques would correlate with each other. The results of this thesis research support the notion that eye movement control tasks, DTI and psychometric tests can be used to differentiate children with FASD from typically developing controls. In addition, specific psychometric measures of inhibition, working memory and visuospatial processing were found to correlate with eye movement task outcomes that assessed the same cognitive domains. Similarly, eye movement task outcomes that assess response inhibition correlated to DTI measures in a specific region of the corpus callosum. Overall, these results support the notion that outcome measures obtained using eye movement control tasks may be a valuable functional biomarker of the brain injury induced by prenatal alcohol exposure.

Eye movement scores were extensively investigated in this study. Due to the large sample size, a very detailed analysis of several different outcome measures, including behavioural and metric measures, could be performed and compared across the two groups. The behavioural measures that successfully differentiated children with FASD from typically developing control
children include corrective saccades, direction errors, timing errors, sequence errors, SRT, and anticipatory saccades. Metric measures which successfully differentiate controls from children with FASD include saccade endpoint, peak velocity, amplitude, duration, deceleration, and velocity skewness. Interestingly, some of these measures also differentiate males and females with FASD as each sex appears to display different deficits. Therefore, eye movement control measures appear to be a valuable tool for assessing children with FASD.

The psychometric testing used in this thesis assessed several different cognitive domains. These include inhibition, attention, working memory, visuospatial processing, and set shifting. All psychometric tests conducted found differences between the FASD and control group. While many neurobehavioral deficits have been described, a unique neurobehavioral profile for FASD remains difficult to elucidate. These tests are currently the ‘gold standard’ used in the diagnostic assessment of children with FASD, and therefore it was important to compare eye movement scores to these ‘gold standard’ measures to determine whether the different tests are assessing the same cognitive domains.

Finally, DTI was used to identify specific white matter tracts affected by prenatal alcohol exposure as well as the relationship between the integrity of these tracts and cognitive functioning. The corpus callosum has consistently been found to be negatively affected in children with FASD, and therefore this structure was targeted for examination using manual tractography. The mean diffusivity from the posterior portion of the corpus callosum was found to be significantly greater in the FASD group compared to controls. Additionally, the FA of this same region was found to be significantly correlated to the response inhibition measure (direction errors) obtained in the antisaccade task. Therefore, this result points to the posterior
portion of the corpus callosum as an important area that is impacted by prenatal alcohol exposure that plays a role in behavioural inhibition.

The large sample size obtained also allowed for a thorough investigation of many demographic and subtle deficits in children with FASD. This has illustrated the importance of investigating mitigating variables such as participant sex when analyzing eye movement measures. This analysis also highlights that FASD subtype, SES, medication, comorbidity, and ethnicity all appear to have minimal impact on eye movement control measures identified in this thesis. The psychometric tests and eye movement tasks point to specific overlapping brain regions damaged by prenatal alcohol exposure. Due to the extensive research and brain mapping from previous research in eye movement control, these tasks can be leveraged to better understand what is being assessed by psychometric testing. Additionally, the DTI measures can also be used to run hypothesis driven correlations based on known areas of the brain involved in inhibition.

7.2 Clinical Relevance

The results of this thesis have important clinical implications. One of the original goals of this thesis was to investigate if eye movement tasks are a viable option as a screening or adjunct tool in the assessment of FASD. This is important because FASD is a heterogeneous group of disorders for which there is currently no standardized screening technology in Canada (Chudley, Conry, Cook, Loock, Rosales, & LeBlanc 2005). As previously mentioned, an FASD diagnosis requires a large time commitment and the involvement of many different professionals which makes it time consuming with limited availability to remote communities. Further, total expenditures on FASD diagnostic services in Canada are estimated to range from $3.6 to $7.3 million per year (Thanh & Jonsson 2009). Therefore, there is enormous potential to reduce the
socioeconomic burden of FASD in Canada through effective and efficient screening for diagnosis, along with innovation in delivery of therapy to reduce costs or provide treatment opportunities. This includes recognition and treatment for underlying brain dysfunction, which may in turn translate into improving other problems faced by these children. Eye movement control tasks not only have the potential to recognize these deficits but also to do so at an early age leading to earlier diagnosis and better outcomes. The eye movement control tasks used in this thesis appear to be appropriate for screening as the three tasks take less than one hour to administer, can simultaneously measure multiple cognitive domains, and correlate to psychometric tests currently used in the diagnostic process. Using eye movement control tasks as a screening tool can also help with early identification of those in need of a follow-up assessment or interventions while waiting for a diagnostic assessment. This could then lead to earlier interventions and potentially less adverse outcomes for those prenatally exposed to alcohol (Streissguth, Bookstein, Barr, Sampson, O'Malley, & Young 2004).

7.3 Limitations

There are several limitations with the study population used in this thesis. There was an imbalance between the FASD and control groups on several demographic variables including ethnicity, socioeconomic status and drug therapy. The FASD population consists of about half First Nations individuals but the control group included only one First Nations individual. This imbalance needs to be corrected in future studies to properly represent the First Nations populations in the control group. The socioeconomic status was also significantly lower in the FASD group compared to the control group. This also needs to be corrected in future studies to include children from lower income and education families. Finally, over half of the children with FASD are currently on drug therapy, but the control population was not on any drug
therapy. While this is an imbalance, it is not possible to correct this as the control population by definition had to have no disorders or diagnoses to participate. While the previous demographic variables were not matched between the FASD and control groups, the effect of these variables on the data was investigated for each study and no differences were found.

There was also a higher proportion of children from the FASD group who experienced early life adversity and different living arrangements than the controls. Many children with FASD face early life adversities including abuse, poor attachment, institutionalization, neglect, etc. This can lead to an increased stress response as well as many mental health issues (Fisher et al. 2012; Rasmussen et al. 2008). The control group is much less likely to undergo the same early life experiences; therefore, we are not able to conclude that the deficits found in the current thesis are entirely due to prenatal alcohol exposure as some could be caused by this early life stress. Additionally, many of the children with FASD are also in foster care homes which may not provide the best environment for recovering from this adversity (Steinhausen et al. 1994). The control group did not include any children in care, and therefore potential contributions of this environmental confound to the behavioural and cognitive deficits found in this study cannot be eliminated.

Finally, maternal drinking behaviour during pregnancy was unknown. This is mainly due to poor records and living arrangements of the children. Many medical records are missing this information because it was either not asked or the information is unreliable. As many of these children are in foster care with unknown prenatal alcohol exposure levels, they are often identified as a possible case of FASD during early childhood (around age 5) when cognitive and behavioural issues become more apparent. At this point, determining maternal alcohol use is either difficult to obtain due to the mother’s absence and/or lacks accuracy (Del Boca & Darkes...
2003). Therefore, we were unable to determine the effects of timing and levels of prenatal alcohol exposure on all the measures obtained.

7.4 Future Directions

7.4.1 Computer modeling

To better characterize eye movement control measures, computer modeling could be used to test for the sensitivity and specificity of these tasks. Over 100 different measures can be obtained from the three eye movement tasks used in this thesis. Therefore, it would be useful to train a classifier to identify key measures that, together, are most effective at differentiating children with FASD from controls. This method is not only objective but can also be automated and therefore can be time- and cost-effective for large population screening. This method also has the potential to take into account sex and subtype differences which in turn can provide a more precise diagnosis.

7.4.2 Diagnostic tool in clinics

The current thesis included children who had already been assessed and diagnosed with an FASD; however, to properly evaluate eye movement control tasks as a screening tool, a prospective study needs to be completed. This would involve testing children who have been referred to FASD clinics for diagnosis, in parallel with the current method of diagnostic assessment. Since not all children who are referred for an assessment receive a diagnosis of FASD, this method would be a more robust test to determine whether eye movement control tasks will correctly identify those children who do go on to receive a diagnosis. This could provide a potential breakthrough for diagnosis by providing a high-throughput, low-cost procedure for assessment.
7.4.3 Drug effects

Many children with FASD are on a variety of drugs including stimulants, antipsychotics, antidepressants, anxiolytics, etc., with some on multiple drugs. However, many of these drugs were not developed for or tested on children and the effect they have on brain development is unknown. A longitudinal study examining the effects of these drugs on these children can be conducted to measure any functional and structural brain changes that occur. This can be accomplished by obtaining baseline measures before drug treatment begins and testing at several time points after the onset of drug therapy. This could be used to answer many research questions. First, it can test the efficacy of the drugs on these children by comparing behavioural measures obtained from the eye movement and psychometric tests. Second, the effect these drugs have on the developing brain can be assessed by examining white matter measures such as FA and MD.

7.4.4 Correlations to other MRI data

The current thesis only investigated DTI measures obtained from the corpus callosum. Future studies could investigate other well characterized white matter tracts in children with FASD. Group differences in the metrics of the DTI data can be completed to identify other regions of the brain affected by prenatal alcohol exposure. Correlations with the eye movement and psychometric scores can also be run to identify other white matter tracts involved in the various cognitive domains assessed. This can better characterize the damage prenatal alcohol exposure has and the functional outcomes of this damage.

Additionally, many different brain regions have been hypothesized to be damaged based on the functional measures obtained from the eye movement and psychometric tests conducted in the current study. Structural MRI measures such as cortical thickness and volume measures of
subcortical structures could be used to test some of these hypotheses by running correlations between these measures and the functional scores. This would be especially useful with the eye movement measures as the circuitry has been well elucidated which would provide very specific hypothesis driven correlations. This is important when using such a large dataset as the p-value can be kept relatively large (i.e. p<0.01) when accounting for multiple comparisons.

7.4.5 ADHD

Finally, ADHD is a very common comorbidity in children with FASD. Although we have found no differences between children with FASD with or without this comorbidity, a study should be conducted that compares children with FASD with or without ADHD to children who only have ADHD. This will allow similarities and differences between FASD alone, FASD with ADHD and ADHD alone to be investigated. As ADHD and FASD can be misdiagnosed, this can be used to further enhance the sensitivity and specificity of the measures used in this study. It will also help with differential diagnoses and a more specific screening tool.
Chapter 8 References


Hollingshead, A. A. Four-factor index of social status. 1975.

Hollingshead, A.A. 2011. Four factor index of social status. Yale Journal of Sociology, 8, 21-51


