SACCADIC EYE MOVEMENTS AND EXECUTIVE FUNCTION IN CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDERS (FASD): RESULTS FROM A MULTI-CENTERED STUDY

By

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ABSTRACT

Saccadic eye movements and executive function in children with fetal alcohol spectrum disorders (FASD): Results from a multi-centered study. Ph.D. Thesis, Queen's University, Kingston, Ontario, Canada, August 2008.

A serious consequence of maternal consumption of alcohol during pregnancy is the fetal alcohol syndrome (FAS): characterized by growth deficiency (both pre- and post-natal), craniofacial dysmorphology and central nervous system (CNS) dysfunction. However, in the absence of the characteristic facial features, and without confirmed history of alcohol exposure, clinical diagnosis remains a significant challenge. Recently, the term fetal alcohol spectrum disorders (FASD) has been adopted to encompass all diagnoses relating to a history of prenatal alcohol exposure. The purpose of this study was to test the following three general hypotheses: Children with FASD 1) demonstrate specific deficits in oculomotor control that can be measured using saccadic eye movement tasks, 2) display specific deficiencies in multiple domains of executive function that can be determined using standardized neuropsychological tasks, and 3) reveal deficits in oculomotor control that correlate with deficiencies in executive function as measured using standardized neuropsychological tasks. A preliminary study revealed significant deficits in saccadic eye movement tasks and provided the foundation for a large, multi-centered study assessing oculomotor control and neuropsychological function in children with FASD. A mobile laboratory was created, which facilitated recruitment of 92 control subjects and 89 subjects with FASD. We found significant evidence for oculomotor deficits across multiple outcome measures following the saccadic eye movement

experiments, especially for oculomotor tasks that probe aspects of executive function. Additionally, children with FASD exhibited performance deficits in neuropsychological tasks that assess planning, attention, spatial working memory and strategy; cognitive skills also included within the domain of executive function. Finally, significant correlations between these two objective measures were found for children with FASD, which were not evident in the control sample. These findings are consistent with significant frontal lobe dysfunction. This is an exciting area of research that may hold promise in developing effective screening tools that can assist in the diagnosis of individuals with a history of prenatal alcohol exposure.

CO-AUTHORSHIP

The research described in this thesis was conducted by Courtney Green in collaboration with Alanna Mihic under the supervision of Dr. James Reynolds and Dr. Douglas Munoz, who conceived the studies described herein. Courtney Green conducted and analyzed all eye movement experiments described in Chapters 2 and 3; conducted neuropsychological testing and analyzed all data described in Chapter 4; performed all data analysis described in Chapter 5; collected all Demographic, Connors' Rating Scale and Questionnaire information and performed analyses where relevant (see Appendix F), and wrote the first draft of each Chapter in the thesis. Alanna Mihic assisted with the collection of CANTAB data.

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LIST OF ABBREVIATIONS AND SYMBOLS

ADHD	Attention-deficit hyperactivity disorder
ANOVA	analysis of Variance
ARBD	Alcohol-related birth defects
ARND	Alcohol-related neurodevelopmental disorder
BOLD	blood-oxygen-level dependent
CANTAB®	Cambridge Neuropsychological Test Automated Battery
CDC	Centers for Disease Control and Prevention
cd/m^2	one candelas per square meter
CNS	central nervous system
CV	coefficient of variation
dlPFC	dorsolateral prefrontal cortex
EOG	Electrooculography
FAE	(Possible/Suspected) Fetal alcohol effects
FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorders
FEF	frontal eye fields
FP	fixation point
$GABA_A$	γ-aminobutyric acid receptor type A
Hz	hertz
IOM	Institute of Medicine
IQ	intelligence quotient
in utero	in the uterus
LED	light-emitting diode
MRI	magnetic resonance imaging
ms	millisecond
MTS	match to sample visual search
PEF	parietal eye fields
pFAS	partial fetal alcohol syndrome
PPC	poster parietal cortex
RTI	reaction time
s	second
SC	superior colliculus
SCi	intermediate layers of the superior colliculus
SEF	supplementary eye fields
SEM	standard error of the mean
SOC	stockings of Cambridge
SRT	saccadic reaction time
SWM	spatial working memory
T	target
TMS	transcranial magnetic stimulation
TS	Tourette syndrome
vs	versus
%	percentage
$	dollar

/	per
<	less than
~	approximately
±	plus or minus
®	registered trademark
°	degree
=	equal
α	alpha

Chapter 1 GENERAL INTRODUCTION

Fetal Alcohol Syndrome is 100% preventable. Then why does it still continue?

1.1 Statement of the Research Problem

A serious consequence to maternal ingestion of alcohol during pregnancy is the fetal alcohol syndrome (FAS), which is characterized by growth deficiencies, cranio-facial dysmorphology and central nervous system (CNS) dysfunction (Clarren and Smith, 1978). In the absence of all three diagnostic features, individuals affected by prenatal exposure to alcohol receive different diagnoses based on the symptoms they manifest. Over the years, a variety of diagnostic guidelines have been established that demonstrate the adverse effects of prenatal alcohol exposure on fetal development. Despite these collective efforts aimed at improving public awareness, alcohol consumption during pregnancy continues to play a major role in the prevalence of mental retardation in North America. In 2005, the term fetal alcohol spectrum disorders (FASD) was adopted as an umbrella term and is used to identify individuals with a history of prenatal alcohol exposure, but who may or may not present with all three diagnostic sequelae (Chudley et al., 2005). This term is intended as identification, but does not serve as a definitive diagnosis, though this point is often confused. Although exhaustive attempts at simplifying and unifying the diagnostic processes available to researchers and clinicians have been made early identification still remains a significant challenge. This current limitation served as the foundation for the research project.

The following sections examine the evolution in diagnostic approaches for FASD, with a particular focus on the current systems most widely adopted today in clinical and research practice. Following these guidelines, selected studies that propose techniques

for assessing the CNS dysfunction and craniofacial dysmorphology will then be reviewed. In order to conduct a comprehensive review the search engine PubMed was used in conjunction with the following search criteria within the field "Title/Abstract" and limited to "Humans, English": "fetal alcohol" and "diagnosis"; "fetal alcohol" and "diagnoses" and "fetal alcohol" and "diagnostic". Collectively these searches retrieved approximately 300 publications. Articles were then selected that provided insight into two broad categories: 1) *Diagnostic systems, guidelines and codes*; and 2) *Diagnostic approaches to FASD*. Additional publications were included based on their applicability to these categories.

1.2 Diagnostic Systems, Guidelines and Codes

Although fetal alcohol syndrome is often considered a relatively "new" diagnostic term, the association between prenatal alcohol exposure and adverse postnatal outcomes has been well documented in early writings dating back to Aristotle, the Old Testament and ancient Greece [see reviews (Manning and Eugene, 2007;Calhoun and Warren, 2007)]. Fortunately, over the last 40 years, the role of alcohol as a teratogen has been widely studied and significant advancements have been made to our understanding of alcohol teratogenicity. Though the exact quantity, duration and frequency of exposure that gives rise to birth defects is not known and cannot be determined, alcohol is now a widely recognized teratogen.

While early literature makes reference to FASD, it is a French pediatrician by the name of Dr. Paul Lemoine, who is credited with the first clinical documentation correlating prenatal alcohol exposure to birth defects [French version: (Lemoine et al., 1968); English version: (Lemoine et al., 2003)]. In this publication, there were no

diagnostic guidelines identified, and unfortunately, the significance of his observation failed to make an impact in North America until 1973, when a group of dysmorphologists also noted the correlation. Drs. Kenneth Jones and David Smith published 3 seminal articles reporting children of alcoholic mothers, who presented with similar phenotypic anomalies, and introduced the terminology "fetal alcohol syndrome" (FAS) (Jones et al., 1974;Jones et al., 1973;Jones and Smith, 1973). While these accounts were primarily case studies and representative of extreme situations, they also set the foundation for our current diagnostic guidelines (i.e., the characteristic facial dysmorphology, growth restriction and CNS dysfunction). From this point onwards, a variety of terminologies and techniques were introduced to classify individuals, who present with alcohol-related disorders, in attempt to provide unified diagnostic guidelines for clinical practice. While these advancements dramatically improved upon the general understanding of FASD pathophysiology, the challenges associated with implementing a set of universal guidelines continues to be problematic.

1.2.1 Early Diagnostic Guidelines

Following the initial diagnostic guidelines for FAS set forth by Jones and Smith in 1973, it was clear that the use of a standardized definition for FAS was not well adopted in the medical and research community. In 1975, they further described the characteristic features of FAS and provided pictorial images of affected children from 3 different ethnic groups (Jones and Smith, 1975). These images served to emphasize the fact that FAS was not unique to a given culture. In this account, they also described the first autopsy of a child with FAS, with particular emphasis on the neurological findings. Most notably, they found aberrant neuronal migration resulting in a smooth outer

appearance due to sheets of neuronal and glial cell misplacement over the surface of the cerebrum. Cellular disorganization was also found and incomplete development was evidenced by the enlarged lateral ventricles. Agenesis of the corpus callosum was also noted. Interestingly, prenatal growth deficiency was more pronounced with respect to length than weight; which was in direct contrast to generalized maternal under-nutrition, in which newborns are underweight for their length. It was concluded that while malnutrition may exacerbate the situation, it was not the major contributor to these postnatal symptoms. These early accounts also make reference to a continuum of adverse effects resulting from prenatal alcohol exposure, and suggested considerable variability in the severity of malformation and dysfunction in the offspring of alcoholic women. The similarity in pattern of anomalies suggested a single etiology: ethanol.

In 1978, Clarren and Smith provided further insight into the distinguishing features associated with FAS (Clarren and Smith, 1978). From extensive animal and human studies, the teratogenic effects associated with alcohol consumption during pregnancy were appreciated, and the common variant in the ingestion histories of women known to produce affected offspring was alcohol. In their account, Clarren and Smith also make reference to the variability in severity, and based on the cases at that time, a wide spectrum of adverse effects to the developing fetus were noted. The abnormalities associated with alcohol teratogenicity were grouped into four major categories: central nervous system dysfunction; growth deficiencies; characteristic facial dysmorphology; and variable major and minor malformations. A diagnosis of FAS was given only when individuals presented with the three former abnormalities, recognizing that further knowledge was required for less complete expressions. In these situations, individuals

were given the identification "suspected fetal alcohol effects" (FAE). Of the given diagnostic criteria, it was the 'FAS face' that most distinguished these individuals; and in its absence, it was significantly harder to make an alcohol-related diagnosis.

While these guidelines added to the existing diagnostic foundation, a need for further clarification was warranted. To address this issue, the Fetal Alcohol Study Group at the Research Society on Alcoholism meeting in 1980 developed a standardized set of diagnostic guidelines for FAS (Rosett, 1980). After reviewing the 245 cases studies reported by Clarren and Smith (Clarren and Smith, 1978), the study group recommended that a diagnosis of FAS be made when a patient demonstrated signs in each of the following three categories: (A) prenatal and/or postnatal growth retardation (weight, length, and/or head circumference below the 10^{th} percentile when corrected for age); (B) central nervous system dysfunction (signs of neurological abnormality, developmental delay, or intellectual impairment); and (C) characteristic facial dysmorphology with at least two of 3 signs: microcephaly (head circumference blow the 3^{rd} percentile), microophthalmia and/or short palpebral fissures, or poorly developed philtrums, thin upper lip, and flattening of the maxillary area. Although the FAS facial phenotype is specific to alcohol teratogenicity, it was apparent that not all individuals presented with craniofacial dysmorphology. In recognizing the wide spectrum of effects, it was suggested that FAS should reside at the far (most severe) end of the continuum, and that in the absence of all 3 diagnostic features, the term FAE should be used.

In 1984, a Hungarian group devised a semiquantitative scoring system for the evaluation of symptoms associated with FAS (Vitez et al., 1984). The total study population was comprised of 587 children of 409 alcoholic women, of which 549

children were recruited into the study. These children were assessed in their local pediatric clinic, and these complex examinations were further evaluated using a "diagnostic scheme of FAS". The schemata included the assessment of symptoms related to brain damage, particularly motor dysfunction, as well as observations of the child's behaviour, psychological status (intelligence quotient), social maturity and personality. Anthropological examinations were also conducted and included measurements of head circumference, weight and stature; and two portrait photographs were assessed for 7 facial characteristics (including palpebral fissure). In total, they studied 60 traits associated with FAS, which fell under 5 major categories: central nervous system, pre- and postnatal growth, face, minor anomalies and major anomalies. A score ranging from -15 to +15 was associated with each unit of measure for each trait, such that negative scores were indicative of children with a more probable diagnosis of FAS. Based on their analysis, the following two points of discriminative value emerged: 1) the diagnosis of FAS should be suspected for scores below -10; and 2) FAS was established for scores below -30. Scores above -10 precluded the diagnosis of FAS. The authors defined established FAS as "typical" and suspected as "atypical". Finally, the authors were able to differentiate the subjects based on their prenatal exposure to alcohol as follows: 1) Drinkers: mothers who consumed alcohol during pregnancy; 2) Abstinent: mothers who drank before or after pregnancy, or both before and after pregnancy; and 3) Control: matched offspring who had randomly chosen mothers. Of the offspring in the "drinkers" cohort, 25 were diagnosed with typical FAS and 168 with atypical FAS. Interestingly, one-third of these children had scores above -10 precluding a FAS diagnosis. This latter point serves to further illustrate the difficulty in generating a set of guidelines that are

appropriate for all situations in which there is a known history of prenatal alcohol exposure.

In 1987, the Fetal Alcohol Study Group once again met at the annual Research Society on Alcoholism meeting and decided that the existing guidelines set-out in 1980 required re-evaluation and the need for related diagnosis was warranted; especially, given the growing body of literature and research under investigation at that time (Sokol and Clarren, 1989). It was necessary to recognize that diagnostic terminology had to evolve with the increased knowledge arising in the field. It was recommended that the newest guidelines supersede the existing criteria published in 1980 and address the following goals:

- *Provide a set of guidelines for use by investigators, care providers and others.*
- *Adherence to guidelines should contribute to clarification in the literature and facilitate the understanding of mechanisms related to alcohol teratogenicity.*

Under these new guidelines, the FAS criteria were retained, requiring the existence of abnormalities in each of the three categories (i.e., growth, CNS dysfunction and facial dysmorphology). Clinicians and investigators were instructed to make detailed notes related to each category, as well as any additional anomalies that would add precision and provide a means for comparisons in the literature. The second term that was introduced was Alcohol-Related Birth Defects (ARBD) and was suggested to describe situations in which an individual presented with anatomical or functional outcomes related to prenatal alcohol exposure, but who did not meet the criteria for FAS. Finally, the term Possible

Fetal Alcohol Effects (FAE) was revisited, and found to be significantly problematic in its use. The committee felt that FAE was ambiguous, and no acceptable definition could be found. Thus, under the implementation of these guidelines, its usage in publication and practice was strongly discouraged.

In 1989, another set of revised diagnostic criteria for FAS appeared in the literature, also recognizing the need for further clarification (Burd and Martsolf, 1989). This criterion has been termed the "Fetal Alcohol Syndrome Checklist". In noting the artificial dichotomy in the usage of FAE and FAS, the authors suggested one inclusive categorization system for FAS, where the full range of associated symptoms attributed to alcohol ingestion during pregnancy were described and scored. In this system, the diagnostic features were divided into major and minor criteria and given scores according to the prevalence of each feature. Cognitive abilities, growth and facial features were considered major criteria, and had weighted scores of 0-3. Minor criteria included a variety of associated abnormalities (i.e., cardiovascular, renal, respiratory, dermatologic or connective tissue) and, if present, were given a score of 1. A total score of 10 points from the major category plus any 2 features under the minor category satisfied their criterion for a FAS diagnosis. It was felt that this system could more appropriately accommodate new symptoms as they emerged into their existing schemata. However, the existence of other alcohol-related diagnoses was not included in situations when the criteria for FAS were not met.

1.2.2 Current Diagnostic Guidelines

Since the first reported cases of alcohol teratogenicity, the evolution in diagnostic guidelines has been appreciated. Today, many of the older methodologies have been

further explored and currently there are 5 sets of guidelines that are most readily adopted for clinical and research purposes. They include: the Institute of Medicine (IOM) FASD guidelines in 1996 (Stratton et al., 1996), the FASD 4-digit Diagnostic Code in 2000 (Astley and Clarren, 2000), the Centers for Disease Control and Prevention (CDC) FAS guidelines in 2004 (Centers for Disease Control and Prevention, 2004), the Canadian FASD guidelines in 2005 (Chudley et al., 2005) and the Hoyme FASD guidelines – Revised IOM criteria in 2005 (Hoyme et al., 2005). As mentioned previously, it is the facial dysmorphology that is most unique to FASD, as growth restriction and neurological damage are associated with other pathologies. However, as seen in Table 1.1, a number of dysmorphic syndromes display similar features, providing further confusion for the appropriate clinical diagnosis. This observation also serves to further emphasize the need for a comprehensive set of diagnostic guidelines that can be easily adopted into the clinical practice and for research purposes.

After the term FAS was introduced (Jones and Smith, 1973), a number of cases arose in which children born to alcohol-abusing mothers did not present with the classic three features. While the continuum of effects was appreciated in early reports, definitive diagnostic criteria for these cases were not identified. As the mechanisms for alcohol teratogenicity remain to be fully elucidated, it has been demonstrated that alcohol can exert its effects through multiple pathways and processes (Cohen-Kerem and Koren, 2003;Goodlett and Horn, 2001); and the timing, dose and frequency of alcohol consumption are all known to contribute to the final manifestations (Riley and McGee, 2005). Although the term 'Suspected Fetal Alcohol Effects' was proposed in 1975, it

Table 1.1 Syndromes with similar phenotypic features to FAS [modified from (Chudley et al., 2005;Manning and Eugene, 2007;Centers for Disease Control and Prevention, 2004;Hoyme et al., 2005)].

Syndrome	**Overlapping Features with FAS**	**Features that are distinct from FAS**
Aarskog syndrome	Widely spaced eyes, small nose with anteverted nares, broad philtrum and midface hypoplasia	Round face, downslanted palpebral fissures, widow's peak, prominent "lop" ears, and specific contracture of digits on extension. Inherited as an X-linked train. Molecular defect identified
Blepharophimosis syndrome (BPES)	Short palpebral fissure and ptosis (droopy eyes)	Epicanthus inversus (skin arising from the lower eyelid and running inwards and upwards), telecanthus (lateral displacement of the inner canthi with normal interpupillary distance) and variable female infertiliy. Inherited as autosomal dominant trait. Molecular defect.
DeLange syndrome	Long smooth philtrum, thin vermilion border of upper lip, depressed nasal bridge, anteverted nares and microcephaly	Single eyebrow across eyes and forehead, long eyelashes, downturned corners of mouth, short upper limb (particularly ulnar side), and very short stature. Molecular defect identified.
Dubowitz syndrome	Short palpebral fissures, widely-spaced eyes, epicanthal folds, variable ptosis and blepharophimosis, microcephaly	Shallow suprorbital ridges, broad nasal tip, clinodactyly
Fetal anticonvulsant syndrome	Widely-spaced eyes, depressed nasal bridge, mid-facial recession, epicanthal folds, long philtrum, thin vermilion border of upper lip	Bowed upper lip, high forehead, small mouth
Maternal phenylketonuria effects	Epicanthal folds, short palpebral fissures, long poorly formed philtrum, thin vermilion border of upper lip, microcephaly	Prominent glabella, small up turned nose, round face

Noonan syndrome	Low nasal bridge, epicanthal folds, wide spaced eyes, long philtrum	Down-slanted palpebral fissures, wide mouth with well-formed philtrum, proturding upper lip. Molecular defect identified.
Toluene embryopathy	Short palpebral fissures, mid face hypoplasia, smooth philtrum, thin vermilion border of upper lip, microcephaly	Large anterior fontanelle, hair patterning abnormalities, ear anomalies
Williams syndrome	Short palpebral fissures, anteverted nares, broad philtrum, maxillary hypoplasia, depressed nasal bridge, epicanthic folds, microcephaly	Wide mouth with full lips and pouting lower lip, stellate pattern of iris, periorbital fullness, connective tissue dysplasia, specific cardiac defect of supravalvar aortic stenosis in many. Chromosome deletion on FISH (fluorescence in situ hybridization) probe analysis of 7q11.23
Other chromosome duplication/deletion syndromes	Many have short palpebral fissures, midface hypoplasia and smooth philtrum	Chromosomal analysis by standard analsysis and some select syndromes by specific FISH probe analysis

was not intended as a definitive diagnosis. Despite this, it was however adopted as such and in 1996 the IOM attempted to further define the term "effects" (Stratton et al., 1996). In this system 5 diagnostic categories were introduced: FAS with (category 1) and without (category 2) a confirmed history of alcohol exposure, partial FAS (category 3), alcohol-related birth defects (ARBD) (category 4) and alcohol-related neurodevelopmental disorder (ARND) (category 5) (Appendix A) in attempt to answer the following 6 questions:

- *Is prenatal alcohol exposure required for a diagnosis of FAS?*
- *Which physical features should be used to define the disorder?*
- *Can behavioural or cognitive features be used to define the disorder?*
- *Is there a role for ancillary measures (i.e., MRI) in diagnosis?*
- *Can the criteria be applied across the lifespan?*
- *What is the relationship between FAE and FAS?*

With the exception of category 2, all diagnoses required a confirmed history of prenatal alcohol exposure. However, further research was suggested to assess the reliability of these categories and to determine whether confirmed prenatal alcohol history was in fact necessary for a diagnosis of ARBD or ARND. The diagnostic terminology set forth by the IOM provided a means for clinician to clinician communications and for clinician to patient communications. Furthermore, there was a mechanism to further assess the etiology and pathophysiology of alcohol teratogenicity, and to select the appropriate treatment strategies.

From a clinical research perspective, misdiagnosis reduces the power to identify meaningful differences between groups; and non-standardized diagnostic tools preclude

valid comparisons across studies. In 2000, Astley and Clarren developed a set of guidelines using a 4-digit diagnostic code after identifying the following five primary limitations to current systems (Astley and Clarren, 2000):

- *Current guidelines are not sufficiently specific to assure diagnostic accuracy*
- *Lack of objective, quantitative scales to measure and report the magnitude of each diagnostic feature*
- *FAE is broadly used and poorly defined*
- *FAE, ARBD and ARND inappropriately imply a causal link between exposure and outcome*
- *FAS and FAE fail to convey the diversity of disability in these individuals*

The 4-digit diagnostic code was established to reflect the magnitude of the four key diagnostic features of FAS in the following order: 1) growth deficiency; 2) FAS facial phenotype; 3) brain damage/dysfunction and 4) gestational alcohol exposure (Appendix B). The magnitude of each feature is reported based on a 4-point Likert scale with 1 reflecting complete absence and 4 reflecting a strong presence of the FAS feature. There are 256 possible diagnostic codes ranging from 1111 to 4444, with each falling into one of 22 unique clinical diagnostic categories labelled A to V. However, only 9 unique diagnostic outcome categories exist, as the other categories only differ by alcohol exposure. These 9 range from 'no cognitive/behavioural or sentinel physical findings detected' to 'FAS'. This coding system was successfully adapted for use in all Washington State FAS Diagnostic and Prevention Networks, and appears to be one of the most widely used diagnostic tools today.

In 2004, the CDC released their set of FAS diagnostic guidelines (Appendix C) with the primary goal of providing a standardized system for physicians, scientists and researchers (Centers for Disease Control and Prevention, 2004). These guidelines reflected a harmonization of existing diagnostic guidelines, and which attempted to adopt a balance between overly conservative guidelines and inclusive diagnostic practices. The structure that was implemented for the task group included information on all facets of the diagnostic process:

- *General framework for referral and diagnosis*
- *Development of guidelines for physical features and exposure*
- *Development of guidelines for potential CNS abnormalities*

The guidelines outlined by the CDC refer only to FAS, as continued work and research were needed before delineating the diagnostic criteria for individuals with a known history of prenatal alcohol exposure, but who did not met all criteria for FAS. As with the IOM and 4-digit diagnostic code guidelines, in the presence of growth restriction, craniofacial dysmorphology and CNS dysfunction, confirmed prenatal alcohol history is not required for a FAS diagnosis.

In 2005, a subcommittee of the Public Health Agency of Canada's National Advisory Committee on FASD reviewed, analyzed and integrated the current approaches for diagnoses to propose a set of universal guidelines for Canada (Appendix D) (Chudley et al., 2005). At this point multiple systems had been introduced for diagnostic purposes, and from these reviews, the major recommendation from this subcommittee was the necessity for a multidisciplinary approach to assessment. Due to the complexity and range of expression of dysfunction, the subcommittee felt a multidisciplinary approach

was critical for accurate and comprehensive diagnosis and treatment recommendations. The suggested team consisted of the following professionals: coordinator for case management, physician, psychologist, occupational therapist and speech-language pathologist, with additional members included based on the need for specific areas of expertise (i.e., addiction counsellors). The Canadian guidelines set forth by the subcommittee reflected a harmonization of the IOM guidelines and the 4-digit diagnostic code. This translated into the implementation of the 4-digit diagnostic code to describe, assess and measure objectively alcohol exposure, growth, facial features and brain damage; while adopting the terminology introduced in the IOM criteria, with one exception. The ARBD category was removed, as the authors' felt its diagnostic utility was limited, as in the absence of other features of FAS or neurological deficits, it is difficult to attribute alcohol causation (see Table 1.1).

Finally, also in 2005 the Hoyme diagnostic criteria were published, which was described as a clarification of the 1996 IOM criteria (Appendix E) (Hoyme et al., 2005). The authors report 3 major problems with the existing IOM guidelines:

- *Vague, with no specific parameters for diagnosis in each category (related to the facial dysmorphology and behavioural/cognitive phenotypes)*
- *Assessment of the family and genetic history of each child is not addressed*
- *ARBD and ARND are not practically defined in a clinical sense*

The improvements proposed by Hoyme *et al.* (2005) were an attempt to add specificity and make the IOM guidelines clinically applicable to the general paediatric practice. The revisions improved upon the vagueness of the original IOM criteria by defining the degree of growth deficiency and specifying the minor physical anomalies. In addition,

pFAS without confirmed maternal alcohol exposure was added. ARBD and ARND were defined with greater specificity, where the former referred to children presenting with specific structural anomalies (i.e., ARBD), and the latter denoting children with a pattern of behavioural or cognitive abnormalities that could not be explained by family or environmental influences alone (i.e., ARND). The method described focused not solely on prenatal alcohol history, but also on the exclusion of known genetic and metabolic syndromes in order to make the appropriate diagnosis (see Table 1.1). As with the Canadian guidelines, the authors also suggested a multidisciplinary approach, involving the participation of physicians, psychologists and skilled maternal interviewers.

1.3 Diagnostic Approaches to FASD

A number of studies have evaluated the use of various objective and subjective tools to assess their utility as diagnostic techniques that could be used to quantify the brain and behaviour dysfunction and facial phenotype associated with FASD. As many adults and children with FASD do not always receive an early and/or accurate diagnosis, they are precluded from accessing the necessary services and supports they need. In terms of the existing FAS diagnostic criteria (growth restriction, craniofacial dysmorphology and CNS dysfunction), generally it is the facial characteristics that have provided the most valuable and distinguishing feature associated with prenatal alcohol exposure. Many research groups have exploited this observation in an attempt to develop quantitative measures for assessing the FASD face. Traditionally, neurological deficits have appeared too broad to serve any diagnostic purpose alone, especially as not all children with FAS demonstrate recognizable mental retardation. However, tools that can objectively quantify the neurological damage associated with a history of prenatal alcohol

exposure could have added clinical and research benefits, as many individuals do not present with the craniofacial dysmorphology. Additionally, these tools may provide greater diagnostic potential, especially measures that are insensitive to cultural differences, as it is the CNS dysfunction that is more common and most debilitating. Selected tools and techniques for assessing neurological function and craniofacial dysmorphology are discussed.

1.3.1 Assessing Neurological Function: Tools and Techniques

In 1998, the behavioural phenotype associated with fetal alcohol exposure was assessed, recognizing that individuals with FAS and FAE often share similar behavioural profiles (Streissguth et al., 1998). Caregivers and parents were asked to depict these individuals using language that described their characteristic behaviours. The most frequently reported behavioural descriptors were assembled into a list called the "Personal Behaviours Checklist". Of the 68 descriptors included, 36 were chosen and this battery was referred to as the "Fetal Alcohol Behaviour Scale", and comprised a simple count of 'yes' responses completed by a primary caregiver (i.e., parent). The battery was demonstrated to have adequate test-retest reliability, and was uncorrelated with age, sex, race and IQ. Most importantly, the battery correlated with maternal alcohol problems, thus it was reflective of the behavioural phenotype associated with a history of prenatal alcohol exposure, with a good degree of specificity. Paternal alcoholism was also evaluated, and the authors demonstrated no correlation with the battery scores, further supporting its specificity for prenatal alcohol exposure, and not the behavioural consequences of residing in an alcoholic family. The authors concluded that

while more research was necessary, the battery may have utility in screening individuals at risk for a diagnosis within the FASD continuum.

Further studies have recognized the need for tools to assess the CNS dysfunction, as full FAS only represents a small fraction of affected children (Koren et al., 2003). In the majority of cases where prenatal alcohol exposure is known, individuals present with marked cognitive and behavioural abnormalities that are irrespective of facial dysmorphology. These cases are typically classified as alcohol-related neurodevelopmental disorder (ARND). A set of criteria for characterizing the neuropsychological profile of children with ARND has been explored (Greenbaum et al., 2002). Based on literature reviews and information from their support centre, 21 areas of weakness or 'deficits' and 6 areas of strengths or 'assets' were identified as relating specifically to an ARND diagnosis. They comprised the 'ARND Diagnostic Criteria Checklist'. Children in the study were administered a comprehensive battery of age-appropriate neuropsychological tests, after which, the examiner and supervising clinical psychologist independently completed the 'Checklist'. Children were assigned to the ARND group if they achieved a minimum of 60% for 'deficits' and 50% for 'assets'. Interestingly, regardless of other 'physical' signs of prenatal alcohol exposure, there were no differences in the number of problems children with ARND maintained according to the 'Checklist'. This provides support for the fact that children with ARND are adversely affected with respect to brain injury, and this is regardless of the degree of physical dysmorphology. This profile approach was based solely on neuropsychological problems, and not behavioural domains, which requires further investigation.

To address this limitation, and the fact that many children with FASD are co-morbid for attention-deficit hyperactivity disorder (ADHD), Nash et al. (2006) conducted a study to distinguish the behavioural phenotype of children with FASD from typically developing children and children with ADHD (Nash et al., 2006). Co-morbidities often lead to improper diagnosis, as well as prescribed treatment regimens that may not be sufficient to ameliorate all aspects of FASD (Coles et al., 1997). In this study, the Child Behaviour Checklist (Achenbach and Rescorla, 2001) was administered to parents/caregivers, which assesses social competencies and behavioural problems. Previous work by this group, in which children with ARND and controls were compared, demonstrated that 62 of 113 behavioural descriptors showed significant differences between the two groups; and 12 of which differed beyond $p<0.001$. Thus, these 12 items were selected for further analysis, and it was found that children with FASD were strongly differentiated from both typically developing children and children with ADHD. Most notably, while children with FASD exhibited attention deficits and hyperactivity, as did the children with ADHD; the FASD group also displayed a lack of guilt after misbehaving, cruelty and tendency to act young for their age, which was unlike the ADHD group. In addition, children with FASD were more likely to lie and steal than children with ADHD. (Children with FASD were significantly different in all 12 items compared to controls). From these findings the authors proposed a 2-step screening tool, in which Step 1 served to identify the behaviours associated with FASD; and in Step 2 children with FASD were differentiated from children with ADHD. This type of screening tool may provide an appropriate early intervention for families, who have limited access to broad diagnostic services, and/or who reside in remote communities.

Recently, the utility of using a narrative analysis (chronologically told story) for identifying children with FASD has been evaluated (Thorne et al., 2007). Narrative analyses may identify children with communicative impairments that would otherwise be missed using conventional assessment instruments. Typically, the context in which these individuals are tested involves the use of discrete responses at or below the level of single-sentence utterances, which gives rise to variable performances and results. However, children with FASD may have difficulty producing integrated speech that requires them to balance linguistic and social-cognitive task demands (Coggins et al., 2003). The Semantic Elaboration Coding System developed by the author (JCT) implements a framework for narrative analysis based upon cognitive linguistics. The coding system has two functions: (1) employs a linguistic reference to ensure that the concepts are explicitly and uniquely identified; and (2) determines the degree to which semantic concepts are elaborated or well specified in the text. In this study, school-aged children perused a wordless picture book, and then were asked to recount the best story possible while using the picture book as a visual prompt. Transcripts were coded based on the Semantic Elaboration Coding System, which assigns codes for ambiguity (inappropriate reference strategies) and elaboration of concepts. Ten mutually exclusive scoring codes were assigned based on these two parameters, plus a null code, which was given when a word did not fit any of the 10 categories. Children with FASD were more likely to use a picture-bound reference strategy during story-telling compared to controls, which was identified using the ambiguity code: 'ambiguous nominal reference' (code 3). This code describes situations, in which the child ambiguously used a name when attempting to introduce, maintain or reintroduce a concept. This code was easy to

compute and had excellent reliability making it a practical approach for assessing children, who perform well on standardized language tasks, but who demonstrate poor performance during social communication in their daily lives.

1.3.2 Assessing Craniofacial Dysmorphology: Tools and Techniques

In recognizing that the craniofacial features represent the most distinguishing characteristic for a FAS diagnosis, various research and clinical groups have devised intricate systems to quantitatively assess these anomalies. The frequency of 60 measurements were assessed in children with a history of prenatal alcohol (FAS and FAE) and compared to controls, in order to determine the occurrence of these manifestations (Autti-Ramo et al., 1992). In the first assessment during the first year of life, 10 children were identified with typical FAS and 19 with possible FAE. Of these 29 children, 22 demonstrated signs of central nervous system dysfunction at 27 months, suggesting the utility of quantifying minor physical anomalies in early infancy to accurately discriminate children at risk for FAS or FAE. Furthermore, this study demonstrated that recognizing the discriminate facial features associated with a history of prenatal alcohol exposure is critical when diagnosing children with disorders of the central nervous system or growth retardation when the etiology is unknown.

Additionally, it has been proposed that the facial features associated with a history of prenatal alcohol exposure may occur on a continuum, and are not always representative of discrete traits. When individuals manifest the characteristic facial phenotype, this criterion is most useful for diagnosing FAS. However, the use of the facial component is more elusive in less severe cases. Thus, the use of craniofacial anthropometry has been used to assess the phenotype associated with prenatal alcohol

exposure in children, with and without FAS (Moore et al., 2002). Craniofacial anthropometry could provide additional discriminating power such that the face may "predict" the brain in these children. Twenty-one craniofacial measurements were used in the analyses of 100 subjects with prenatal alcohol exposure (41 with FAS; 59 with pFAS) and 31 controls. Of the 21 measurements, 19 were significantly different between the FAS and pFAS groups; and also between the FAS and controls. In contrast, only 7 of the 21 measurements successfully discriminated between the pFAS and control groups. These measurements were not confounded by race or age. Using standardized z-scores enabled the comparison between the study population and a reference population. The results from the FAS group demonstrated that their craniofacial measurements were smaller than both the reference population and the pFAS group; while the pFAS group were intermediate between the reference population and FAS. Thus, these data support the implementation of craniofacial anthropometry as a potential screening tool that can be used to identify individuals at risk for FAS and other alcohol-related diagnoses.

In order to reduce costs and the need for pediatric dysmorphologists, the use of a stereo-photogrammetric method was used to compare FAS facial features in the Western Cape province of South Africa (Meintjes et al., 2002). Forty-four children were photographed and their facial features were measured using the proposed stereo-photogrammetric technique. It was found that facial measurements could be performed with greater consistency from a pair of stereo-photographs than from direct measurements of live subjects. Further, this technique was reproducible and non-specialists achieved consistent results providing further support for the utility of such a tool. The benefits of using computerized anthropometry include decreasing the time

required for assessment for both patient and examiner; and can also provide objective measures of the angles, surface areas, volumes and linear distances pertaining to the craniofacial features of FAS, which can then be used to further improve their diagnostic potential.

Although stereo-photogrammetrics have been used in various studies to assess their sensitivity (percent of FAS subjects correctly classified) and selectivity (percent of control participants correctly classified), only recently have these techniques been applied to differentiate patients with FAS from controls across wide age ranges and across ethnically disparate populations (Moore et al., 2007). Subjects were recruited from 4 distinct regions: North American Caucasian, African American, Finnish Caucasian or Cape Coloured (of mixed ancestry) to determine the unique set of anthropometric features that could be identified in each of the study populations. The facial features that most effectively discriminated FAS and controls differed across the populations, although in each of the 4 study groups, at least one eye measurement (shortened palpebral fissure, reduced outer canthal width or reduced inner canthal width) was included in the model. These findings were consistent with clinical descriptions where the orbital region and midface were the most highly distinguishable features associated with FAS. This study further supports the applicability of anthropometry for identifying individuals with FAS among different ages and ethnicities.

FAS-related disabilities are often compounded by secondary emotional and behavioural disabilities and criminality when the syndrome fails to be diagnosed (Streissguth et al., 2004;Streissguth et al., 1991;Streissguth et al., 1985). With early identification, many of these secondary manifestations can be prevented, or at least their

severity can be reduced. Unfortunately, the lack of efficient and effective surveillance, screening and diagnostic tools significantly compromise the early diagnosis of FAS. Recognizing this clinical limitation, the FAS facial phenotype has been further assessed using facial photographs in attempt to develop a highly efficient, accurate and precise screening, surveillance and diagnostic aide (Astley and Clarren, 1996). Although the facial features associated with FAS have been previously described (i.e., small palpebral fissure, smooth philtrums and thin upper lip (Clarren and Smith, 1978), criteria that clarifies *how* small, smooth or thin these features are had yet to be defined in the literature. In comparing frontal facial photographs from subjects with FAS and controls, stepwise discriminant analysis revealed a cluster of 3 facial features that best differentiated patients with and without FAS; namely, palpebral fissure length/inner canthal distance ratio, philtrum smoothness (measured on a Likert scale), and upper lip thinness (measured on the continuous scale of circularity). Using facial photographs and computer software provided an objective means for differentiating subjects, as this approach maximized accuracy, precision and efficiency; all of which are important in developing effective screening, diagnostic and surveillance tools. Furthermore, this cluster of 3 facial features appeared to be the minimum number required for defining the phenotype and for differentiating individuals with the highest accuracy. This tool provides a standardized technique for reporting the facial anomalies associated with a history of prenatal alcohol, and holds further benefits for comparisons across study populations.

Using the FAS facial phenotype screening tool, the prevalence of FAS in a foster care population has been assessed (Astley et al., 2002). After screening 600 children, it

was confirmed that foster care is a high-risk population for FAS and that assessments using this tool can be performed accurately, efficiently and with direct benefit to the child and family. This study served to highlight the utility of screening in terms of increasing primary and secondary intervention/prevention strategies once a child has been identified. With the successful identification of these children using this screening tool, the child's disability can be documented and service workers can place them in families that are willing to support their needs. Increased awareness and understanding among caregivers and social workers improved their ability to recognize children at risk and seek out diagnostic services on their behalf. Because this tool can be easily implemented in different healthcare centres, families were not required to travel significant distances to obtain diagnostic services. This alleviated significant costs and improved the efficiency with which these children obtained access to the services and supports they required.

Current diagnostic criteria for FAS have been problematic in the identification of newborns, due to the difficulty in assessing neurodevelopmental, cognitive and behavioural patterns. This issue is further compounded by women, who under-report their alcohol use, and many physicians, who are unfamiliar with the characteristic features. In a prospective study, 4 maternal blood markers of alcohol use were compared to self-reporting measures on screening interviews and questionnaires in pregnant women; and together these results were compared to the effects on their infants (Stoler and Holmes, 2004). Affected infants, who presented with evidence of growth retardation and a positive facial score (presence of 4 characteristic features) were considered FAS; while those with either growth retardation or positive face were considered pFAS or FAE. Facial scoring systems may prove to be significantly helpful in assessing babies

with prenatal alcohol exposure, and at the very least, assist in identifying those children at risk for further cognitive deficits so they may be followed more closely.

1.3.3 Assessing Visual and Oculomotor Impairments: Tools and Techniques

The characteristic craniofacial dysmorphology associated with prenatal alcohol exposure has now been well characterized. Interestingly, the orbital cavity and visual system appear to be particularly sensitive to the adverse effects of alcohol. In particular, the eye is a sensitive indicator of harmful prenatal events, and has been useful in the investigation of teratogens, and these opthalmological landmarks may assist in the diagnosis of FAS (Stromland and Pinazo-Duran, 2002;Stromland, 2004). The early development of the eye is known in detail, which means that critical time periods for the developmental of ocular abnormalities can therefore be set using developmental timetables.

In particular, a variety of eye abnormalities have been noted in individuals with FAS [reviews (Stromland and Pinazo-Duran, 2002;Stromland, 2004)]. Refraction varies among children with FAS and ranges from severe myopia (near-sightedness) to moderate hyperopia (far-sightedness). Poor vision in children should be treated as early as possible to achieve good results, and eye examinations are critical for children with FAS. Although strabismus has been associated with FAS, it is a common non-specific finding in ophthalmology. However, in association with other features of FAS it may prove to be a valuable distinguishing feature. Intraocular malformations include asymmetry of the eyes, and can range from subtle, minor isolated lesions of the retina to severe malformations involving more than one structure. It is not surprising that retinal and optic nerve deficits occur in individuals with FASD, as these structures are associated

with developing brain tissue. The most frequent eye abnormality is incomplete development of the optic nerve, which is characterized by subnormal vision and subnormal optic nerve axons. As eye pathology is closely associated with FAS, ophthalmological examinations may assist in the identification of individuals with a history of prenatal alcohol exposure.

Among the FAS facial features, several regions near the orbits can be evaluated quantitatively using distance measurements. Anthropometric analysis is now an area of active research and adaptation of this methodology using photographs that specifically measure the distances around the eye may reduce the time of clinical examinations and improve reliability. Recently, an algorithm that automatically extracts the eye and iris contours in facial photographs has been developed and may be adapted for wide-scale surveillance projects (Douglas et al., 2003). Using this methodology, palpebral fissure length, interpupillary distance and inner and outer canthal distances were assessed to determine the suitability of reference values for diagnosing FAS in children from South Africa (Douglas and Viljoen, 2006). These values were compared with other measurements taken from published reports of different ethnic populations. Interestingly, significant differences in eye distance measurements in black South African children were found when compared to these reports, suggesting that normative data sets must be generated based on the study population. Thus, while eye measurements may assist in the screening of individuals at risk for FASD, there is a need to establish standard reference values for these four measurements that are specific to different ethnic populations. Still, taken together with the visual impairments common to the FASD

population, these measurements may have further utility as a cost-effective screening tool.

1.4 Eye Movement Experiments: Saccades

Eye movement experiments have been a source of valuable information to both clinicians and basic scientists. The study of the eye presents a unique opportunity to understand the workings of the brain, and eye movement control holds several advantages to studying axial and limb movements. These include the fact that several different classes of eye movements exist, which can be distinguished based on their physiological properties, and anatomical substrates. Also, many abnormalities associated with eye movements are distinctive and can provide insight into the particular pathophysiology that may accompany such an abnormality. Additionally, the motor neurons that govern eye movement behaviours are located within the cranium, thus enabling the study of motor function without using body movements. Of the different classes of eye movements, *saccades* are the fastest type of eye movements and are used to redirect the line of sight. Saccadic eye movements represent a superb model system to investigate the ability to selectively control behavioural responses.

A saccade has the main function of bringing images of interest onto the fovea (Leigh and Zee, 1999), and includes both the voluntary and involuntary changes of fixation. A number of different types of saccades exist and are useful for neuro-ophthalmologic examination and research purposes. Of these different saccades, three are most relevant to this research project. *Automatic saccades* (i.e., *prosaccades*) are externally triggered by the sudden appearance of a target on the peripheral part of the retina that occurs unexpectedly within the environment. A unique type of reflexive

saccades is the *express saccades*, which are very short latency saccades that occur following the presentation of a novel stimulus. *Volitional saccades* are internally triggered, elective saccades made as part of purposeful behaviour; and one type is the *antisaccade*, which occurs when saccades are generated in the opposite direction to the sudden appearance of a target (following such instructions).

In the anti/pro task, a fixation point (FP) appears in the middle of a visual display and subjects are instructed to either look towards (prosaccade) or away from (antisaccade) an eccentric visual target (T) that appears in the right or left visual field (Fig. 1.1). Successful performance in the antisaccade tasks requires two sequences of events: 1) suppression of the automatic prosaccade towards the peripheral target; and 2) volitional generation of saccade in the opposite direction. Thus, prosaccades probe the ability of participants to initiate automatic, visually triggered saccades; and antisaccades assess voluntary control and the ability to suppress reflexive saccades. Visually triggered saccades are mediated by the intermediate layers of the superior colliculus (SCi), with important inputs from the visual and posterior parietal cortices (Munoz and Everling, 2004;Schiller et al., 1987); while volitional saccades rely on circuitry that includes higher brain centres such as the frontal cortex and basal ganglia (Hikosaka et al., 2000;Dias and Segraves, 1999;Gaymard et al., 1998;Pierrot-Deseilligny et al., 1991). One's ability to perform the antisaccade task develops during the transition between childhood to adolescence, and adult performance is obtained at approximately 18 years of age (Munoz et al., 1998); and peak performance is noted in the early 20's, after which latency increases with age. Cerebral lesions, particularly involving the frontal lobes, lead to disturbances in antisaccades.

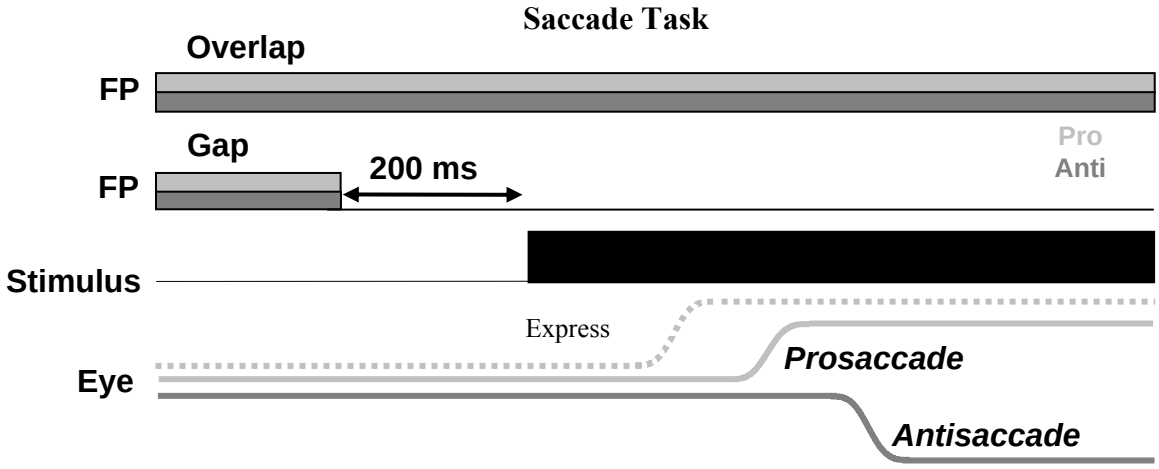

Fig. 1.1. The pro- and antisaccade task. In the prosaccade task the subject was instructed to look from the central fixation point (FP) towards the eccentric target. In the anti-saccade task the subject was instructed to look away from the eccentric target to the opposite side. In both tasks, the state of fixation prior to the saccade was manipulated. In the overlap condition, the FP remained illuminated while the target appeared. In the gap condition, the FP disappeared for 200 ms before the target appeared. In both conditions, the SRT was measured from the time of target appearance to the initiation of the first saccade.

1.4.1 Neurophysiology of Saccadic Eye Movements

There are six extra-ocular motoneurons (MN) that encode the characteristics of the saccade in terms of their temporal discharge. They discharge a burst of action potentials to move the eyes and a tonic discharge to keep the eyes in a fixed position (Leigh and Zee, 1999). The size of the saccade is proportional to the total number of discharge spikes. The MN lie in the third, fourth, and sixth cranial nerve nuclei and cause the extra-ocular muscles to move the eyes with respect to the head, in craniotopic coordinates. Thus, the brain must transform the stimulus in terms of the location of active neurons within the visual cortex into the saccadic command on ocular MN. MN are innervated by *excitatory* and *inhibitory burst neurons* originating from the brainstem reticular formation. In addition to these two classes of neurons, there are the *long-lead burst neurons* and the *omnipause neurons*, which are also located in the brainstem reticular formation. The long-lead burst neurons project to the excitatory and inhibitory burst neurons causing them to discharge; while the omnipause neurons lead to their inhibition. In order for a saccade to occur, the omnipause neurons must be silenced and the long-lead burst neurons must activate either the excitatory or inhibitory burst neuron pools to generate a saccade command to the MN. Once the saccade is complete, the omnipause neurons are reactivated and the excitatory or inhibitory burst neurons are inhibited once more.

The frontal cortex plays a major role in controlling saccadic eye movement, as it receives direct projections from the visual cortex (Fig. 1.2). The frontal eye fields (FEF) interconnect with the parietal visual areas and it has been postulated that this area acts as a central “hub” connecting the supplementary eye fields (SEF), dorsalateral prefrontal

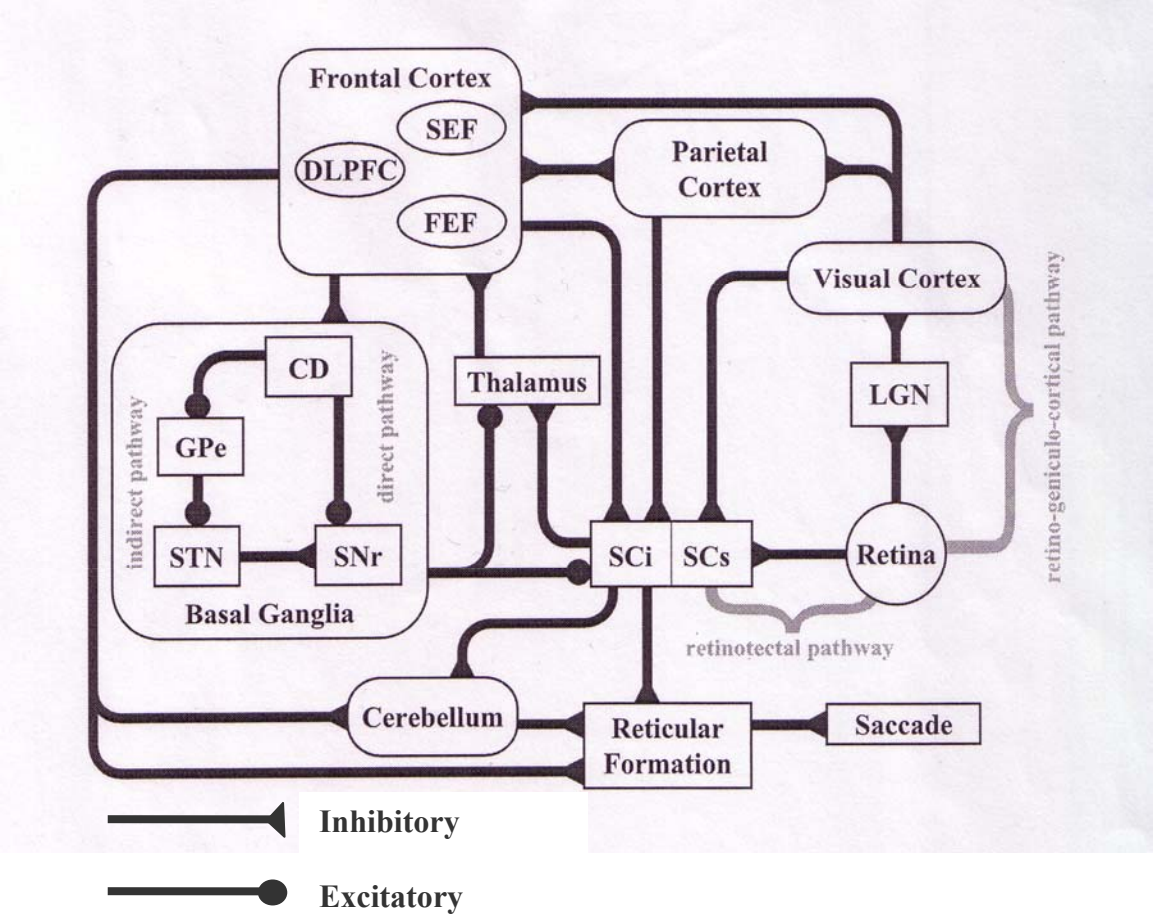

Fig. 1.2 Brain areas involved in saccade control. Visually-triggered saccades (i.e., prosaccades) occur following the sudden appearance of a visual stimulus, and are governed by the SCi, with inputs from the visual and posterior cortices. Volitional saccades (i.e., antisaccades) are generated by internal goals and rely on higher brain centres that include the frontal cortex and basal ganglia [Reproduced with permission (Munoz et al., 2007)]. CD: caudate nucleus; DLPFC: dorsalateral prefrontal cortex; FEF: frontal eye fields; GPe: external segment of the globus pallidus; LGN: lateral geniculate nucleus; SEF: supplementary eye fields; SNr: substantia nigra pars reticulata; SCi: intermediate layers of superior colliculus; SCs: superficial layers of superior colliculus; STN: subthalamic nucleus.

cortex (dlPFC), parietal cortex, SCi and basal ganglia (Munoz et al., 2007;Thompson et al., 1997;Schall and Thompson, 1999). The FEF, parietal lobe and SCi have numerous connections between them, and contain neurons with similar firing patterns. The FEF, SEF and DLPFC all project directly to the SCi; and the FEF and SEF also project to the cerebellum and reticular formation. The frontal cortex also connects to the basal ganglia and participates in presaccadic activity via either a *direct* or *indirect* pathway (Munoz et al., 2007;Hikosaka et al., 2000;Munoz and Everling, 2004). In the *direct* pathway, projections to caudate nucleus lead to the inhibition of the substantia nigra pars reticulata via GABAergic projections. The substantia nigra pars reticulata is the major output centre for the basal ganglia and sends GABAergic projections to both the thalamus and SCi, which in turn project back to the frontal and parietal cortices. As there are two inhibitory synapses involved in the direct pathway, this results in disinhibition of the SCi and thalamus. In the *indirect* pathway, the caudate sends separate GABAergic projections to the external globus pallidus, which in turn sends inhibitory projections to the subthalamic nucleus. The subthalamic nucleus projects to the substantia nigra pars reticulata, which forms synapses with the thalamus and SCi (as in the direct pathway). Unlike in the direct pathway, the addition of a third GABAergic projection leads to the inhibition of the SCi and thalamus. This, in turn sends the appropriate motor command resulting in saccade generation. Thus, dysfunction or immaturity of the frontal cortex and/or basal ganglia may influence the successful execution of saccades, and this can be tested experimentally.

1.5 Research Rationale, Hypothesis and Objectives

This thesis research represents a multifaceted approach to investigating the concordance between deficits in the control of saccadic eye movements and standard neuropsychological tests in children with a diagnosis within the FASD continuum. Importantly, this study reflects the involvement of multiple centres across Canada and the results reflect the participation of over 200 volunteer subjects, families, caregivers and support workers. The aim of this study was to develop a novel and objective approach for further assessing the brain injury associated with a history of prenatal alcohol exposure; with the long-term goal of developing a screening and research tool that could aid in the diagnosis of these conditions, and to evaluate the efficacy of therapeutic interventions. Because saccadic eye movements are easy to assess in children and provide an objective measure of brain function, they are well suited for studying the FASD population. Additionally, the use of standard neuropsychological testing tools that can further quantify the deficits in executive function can be assessed and correlated with distinct outcome measures in oculomotor performance, to give insight into the particular brain regions that are most compromised.

The purpose of this study was to test the following general hypotheses: Children with FASD:

- *Demonstrate specific deficits in oculomotor control that can be measured using saccadic eye movement tasks.*
- *Display specific deficiencies in multiple domains of executive function that can be determined using standardized neuropsychological tasks.*

- *Reveal deficits in oculomotor control that correlate with deficiencies in executive function as measured using standardized neuropsychological tasks.*

The general objectives of this research project were as follows:

- *Assess the feasibility of eye movement testing in children with FASD.*
- *Adapt the oculomotor laboratory for mobile use across different community settings to enable testing across multiple test sites.*

Chapter 2 DEFICITS IN EYE MOVEMENT CONTROL IN CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDERS

2.1 Introduction

The major consequence of prenatal ethanol exposure is fetal alcohol syndrome (FAS) (Astley and Clarren, 2000;Clarren and Smith, 1978;Chudley et al., 2005). FAS is characterized by growth restriction (both pre- and post-natal), craniofacial dysmorphology (i.e., indistinct philtrum, short palpebral fissure) and central nervous systems dysfunction. Recently, the term fetal alcohol spectrum disorders (FASD) has been introduced and widely adopted as an umbrella term, which includes all disorders relating to prenatal alcohol exposure (Koren et al., 2003). Although the terminology has been clarified, an accurate diagnosis still remains a significant clinical challenge due largely to the absence of objective diagnostic tools; and particularly in cases where the craniofacial dysmorphology is absent, but the cognitive deficits are still prevalent.

Executive functions consist of those capacities that enable a person to engage successfully in independent, purposive, self-serving behaviors (Lezak, 1995;Funahashi, 2001), and deficits in these functions are now recognized as a hallmark of prenatal ethanol exposure. Individuals with FASD exhibit a range of deficits in executive function, including problems with flexibility of thought, planning, impulsivity, verbal reasoning, task switching, and working memory (Rasmussen, 2005). Indeed, several studies have documented deficits in executive function in children with a history of prenatal ethanol exposure, but without the facial dysmorphology of FAS (Mattson et al., 1999;Schonfeld et al., 2001;Connor et al., 2000). This finding reinforces the view that of the three key diagnostic features, it is the deficits in brain function that are of primary importance (Chudley et al., 2005). Thus, further research into the specific pattern of

executive function deficits in the FASD population, using objective and consistent measurement tools, is needed (Rasmussen, 2005).

One tool that has been used extensively to study deficits in motor control, working memory, and executive function associated with various neurodevelopmental and neurodegenerative disorders is saccadic eye movements (Munoz et al., 2007;Leigh and Zee, 1999;Leigh and Kennard, 2004;Munoz and Everling, 2004). The underlying neural circuitry that controls saccadic eye movements is now understood to an extent that higher cognitive function can be probed using saccade paradigms, and these tests may be used to assess specific deficits in executive function in patients with FASD. Thus, oculomotor tasks provide a unique opportunity to probe the effects of prenatal alcohol exposure on brain and behavior relationships in a way that other cognitive tasks cannot.

There is considerable overlap in the structures that are responsible for producing a saccade (Leigh and Zee, 1999), and those that are damaged by prenatal alcohol exposure, including the frontal lobes, thalamus, basal ganglia, and cerebellum (Sowell et al., 2002;Mattson et al., 2001;Rasmussen, 2005). Damage to these structures can affect saccade production and these deficits are measurable and quantifiable.

An important feature of human behavior is the ability to respond flexibly to different environmental stimuli. These attributes can be investigated in specific oculomotor paradigms. Subjects can be instructed to look toward a visual stimulus (Fig. 1.1, prosaccade task, requires automatic response) or suppress this automatic response and look away from the stimulus (Fig. 1.1, antisaccade task, requires volitional response)

(Munoz and Everling, 2004;Hallett, 1978). The prosaccade task assesses basic sensorimotor reflexes, as well as the ability to maintain visual fixation, while the anti-saccade assesses voluntary motor control because subjects must suppress the automatic response towards the peripheral stimulus before planning the volitional response (Munoz and Everling, 2004). In addition, to further probe response inhibition, two fixation conditions are frequently used. In the gap condition, a central fixation point is extinguished prior to the onset of the peripheral stimulus. Subjects experience greater difficulty inhibiting the prepotent response under the gap condition. In the overlap condition, the central fixation point remains illuminated while the peripheral target appears, making it easier for subjects to suppress the unwanted movement toward the stimulus. Because cognitive function includes the ability to voluntarily inhibit prepotent responses, guide goal-directed behavior and use working memory, oculomotor tasks can thus be used to dissociate these different cognitive abilities.

Saccadic eye movement experiments have been performed over a wide range of ages, including children (Munoz et al., 1998;Salman et al., 2006;Fischer et al., 1997). This technique is non-invasive and easy to administer making it highly suitable to assess executive function in the FASD population. Furthermore, saccadic eye movement experiments have been used to characterize a variety of neuropsychological and neurodegenerative diseases (Ramat et al., 2007;Munoz et al., 2007), including schizophrenia (Currie et al., 1993;Zanelli et al., 2005), attention-deficit hyperactivity disorder (ADHD) (Munoz et al., 2003;Armstrong and Munoz, 2003;O'Driscoll et al., 2005;Klein et al., 2003), Parkinson's disease (Chan et al., 2005;Le Heron et al., 2005;Crawford et al., 1989;Kimmig et al., 2002), Tourette's syndrome (LeVasseur et al.,

2001) and Alzheimer's disease (Crawford et al., 2005). The existing knowledge from these groups may assist with the identification of traits that are unique to FASD.

The aim of this study was two-fold. The first objective was to determine if saccades could be measured in children with FASD using pro- and antisaccade tasks, as these experiments have not been previously conducted in this clinical population. The second objective was to quantify the control of automatic and volitional responses using pro- and antisaccade tasks. It was hypothesized that subjects with FASD would have no trouble performing saccadic eye movement experiments; however, based on the known deficits in frontal lobe function, these children were expected to produce saccades with increased saccadic reaction times and make more direction errors in the anti-saccade task compared to control children. In addition, because of known brainstem and cerebellar dysfunction, children with FASD were expected to produce significant deficits in saccade metrics (amplitude, velocity and duration). These tasks provide an excellent method for quantifying and comparing objectively the responses of children with FASD to those of control subjects, and may provide insight into the specific types of brain damage associated with prenatal ethanol exposure.

A preliminary version of these data has been presented in abstract form (Green et al., 2006;Green et al., 2004;Green et al., 2005).

2.2 Materials and Methods

2.2.1. Participants

All experimental procedures were reviewed and approved by the Queen's University Human Research Ethics Board. We selected children with FASD, as determined by a clinical geneticist and according to the Canadian diagnostic guidelines (Chudley et al., 2005). Ten children with FASD (four male, six female; 9.8 ± 0.4 years of age, range 8-12 years) were recruited and compared with 12 age-matched control subjects (six male, six female; 10.0 ± 0.3 years of age, range 8-12 years). Of the ten children with FASD, eight were medicated for behavioral symptoms relating to their co-morbidities (Table 2.1). Children were tested off their medication, such that their last daily dose was administered the day before arriving at the lab; recognizing that for some pharmacological agents this was not a sufficient wash-out period. Agents that were most likely to interfere with testing were stimulant medications that have a relatively short half-life (~6-12 hrs) (Katzung, 1998) and for which the overnight wash-out period was sufficient. The contribution of each drug therapy could not be addressed in this study due in part to sample size, but more precisely due to the extensive differences that exist between medication regimens and treatment history. All control subjects had no known neurological, psychiatric, or visual disorders, other than requiring corrective lenses, which were worn if needed throughout the experiments. Parents and/or legal guardians were informed of the nature of the study and provided written consent on behalf of the participants. All subjects completed one, 1-h session and were paid $10.

Table 2.1: FASD subject information.

n	IOM	Sex	Age	Medication	Co-morbidities	Education History
1	pFAS	F	10	-Lithium -Risperidone -Fluoxetine	-Anxiety Disorder -Depression -Bipolar Mood Disorder -Oppositional Defiant Disorder	-Resource help
2	ARND	F	11	-Risperidone -Dextro-amphetamine	-ADHD	-Suspension
3	ARND	M	12	-Risperidone -Dextro-amphetamine	-ADHD -Depression -Oppositional Defiant Disorder	-Suspension -Learning disability -Resource help -Special education -Special program -Special class
4	ARND	F	10	-Risperidone -Clonidine -Valproic Acid	-ADHD -Oppositional Defiant Disorder -Neurological Disorder	-Learning disability -Resource help -Special education -Special class
5	FAS	F	10	-Methyl-phenidate	-ADHD	-Learning disability -Resource help -Special education -Special program
6	FAS	F	10	-n/a	-n/a	-Learning disability -Resource help
7	pFAS	F	9	-Methyl-phenidate	-ADHD	-Suspension -Resource help -Special education -Special program -Special class
8	ARND	M	8	-Methyl-phenidate -Clonidine	-ADHD -Conduct disorder	-Suspension -Learning disability -Special education -Special program -Special class
9	pFAS	M	9	-Methyl-phenidate -Risperidone	-ADHD -Developmental Delay	-Learning disability -Resource help -Special education -Special program -Special class
10	FAS	M	9	-n/a	-ADHD	-Suspension -Learning disability -Resource help -Special education -Special program -Special class

2.2.2 SaccadeTask

All participants performed the saccade task (Fig. 1.1), consisting of one block of prosaccade trials, followed by two blocks of antisaccade trials, each block consisting of 100 trials. Subjects received breaks and refreshments between blocks. Participants were seated in a dentist chair, while in complete darkness facing the centre of a translucent screen located 100-cm away. A red light-emitting diode (LED; 2.0 cd/m^2) was positioned onto the centre of the screen and served as the initial fixation point (FP). Red target LEDs (5.0 cd/m^2) were positioned at 20° to the right or left of the central FP. The screen was diffusely illuminated between trials to avoid dark adaptation. Each trial began with a 250 ms period of complete darkness. The FP appeared for 1000 ms and then one of two events occurred. In the gap condition, the FP was extinguished and, after a gap period of 200 ms, the eccentric stimulus appeared in the right or left visual field. In the overlap condition, the FP remained lit when the eccentric stimulus appeared. In the pro-saccade task, participants were instructed to start each trial fixated on the central FP and then look towards the stimulus as soon as it appeared. In the antisaccade task, participants were instructed to look away from the eccentric stimulus to the opposite side. The stimulus remained illuminated for 1000 ms after which all LEDs disappeared and the background illumination reappeared, indicating the end of that trial. Stimulus location (right or left) and fixation conditions (gap or overlap) were pseudo-randomly interleaved throughout each block of trials. Subjects were asked to repeat the instructions to the experimenter to ensure they understood the paradigm before the onset of data collection.

2.2.3 Recording and Analysis of Eye Movements

Horizontal eye position was measured using DC-electrooculography (EOG). Ag-AgCl electrodes were affixed bitemporally and a grounding electrode was placed in the centre of the forehead. All experimental data were digitized at 1 kHz using REX (ver 5.4; (Hays et al., 1982)) and analyzed off-line on a Sun Ultra 60 Sparc station.

Saccadic reaction time (SRT) was defined as the time from stimulus appearance to initiation of the first saccade that exceeded 30°/s. Saccades were scored as correct if the first movement after appearance of the eccentric stimulus was >5° in amplitude and in the correct direction (i.e., toward the stimulus in the prosaccade task; away from the stimulus in the antisaccade task). Saccades were scored as incorrect if the first saccade after appearance of the stimulus was in the wrong direction (i.e., away from the stimulus in the prosaccade; toward the stimulus in the antisaccade task). Mean SRT in the pro- and antisaccade tasks was computed from trials with reaction latencies between 90 and 1000 ms. These criteria served to eliminate anticipatory saccades and atypically long responses (Munoz et al., 1998). In addition, we measured express saccades (latency: 90-140 ms), which are the shortest latency visually-triggered saccades (Fischer and Ramsperger, 1984;Munoz et al., 1998;Dorris et al., 1997). Express saccades have latencies that approach the minimal afferent and efferent conduction times for visual information to reach the oculomotor system and to be translated into a rapid eye movement (Pare and Munoz, 1996;Dorris et al., 1997). Neurons in the superior colliculus receive inputs from sensory, motor and cognitive inputs, and these inputs contribute to establishing specific levels of excitability among populations of collicular neurons (fixation and saccade) that result in the generation of express saccades.

The following parameters were computed for each condition (gap, overlap) and direction (right, left): the mean SRT for correct trials, the coefficient of variation of SRT for correct trials [(CV = standard deviation/mean) x 100], the percentage of express saccades and the percentage of direction errors. Metric analyses for correct prosaccade trials were also carried out to determine the amplitude of the first saccade and the number of saccades made to reach the stimulus. For correct prosaccades that were restricted to 18°-21° in amplitude, duration and peak velocity were also computed.

2.2.4 Data Analysis

The two experiments (pro- and antisaccade tasks) contained two within-subject factors: fixation state (gap vs. overlap) and direction (right vs. left); and one between-group factor: clinical pathology (FASD vs. control). All dependent measures (SRT, CV, express saccades, direction errors) were analyzed using ANOVA with alpha set at 0.05. Two-tailed, unpaired Student's *t*-tests were conducted and corrected with Welch's approximation when the assumption for homogeneity of variance was not met. We will focus on descriptions of the relevant statistical parameters for comparisons and interactions that occurred between the control and FASD groups.

2.3 Results

2.3.1 SRT

Figure 2.1 depicts the cumulative distribution of reaction times for correct responses (positive values) and direction errors (negative values) for control and FASD children in the pro- (Fig. 2.1A, C) and anti- (Fig. 2.1B, D) saccade tasks in both the gap (Fig. 2.1A, B) and overlap (Fig. 2.1C, D) conditions. Controls were faster to react and initiate saccades in both the pro- and antisaccade trials (solid traces lead dashed traces). The gray boxes in Fig. 2.1 depict the express saccade epoch, 90-140 ms after stimulus appearance. In the prosaccade task, the FASD group made more direction errors and fewer express saccades compared to controls. In the antisaccade task FASD subjects made more direction errors (though not significant); however, these errors were generated at very different times. The majority of direction errors made by controls were triggered in the express saccade range (gray box), while FASD direction errors were triggered later.

The ANOVA revealed the following for dependent measures (SRT, CV, express saccades, direction errors). FASD children had slower SRT compared to controls ($F(1,20) = 20.9$, $p < 0.001$). Consistent with previous studies, mean SRT was increased for antisaccades compared to prosaccades ($F(1,20) = 47.7$, $p < 0.001$), and in the overlap condition compared to the gap condition ($F(1,20) = 94.2$, $p < 0.001$) for all groups. There were no significant directional effects.

Figures 2.2 and 2.3 summarize the data from the pro- and antisaccade tasks, respectively. In both the gap (Figs. 2.2A, 2.3A) and overlap (Figs. 2.2B, 2.3B) conditions, the mean SRT was elevated in the FASD group compared to controls in the

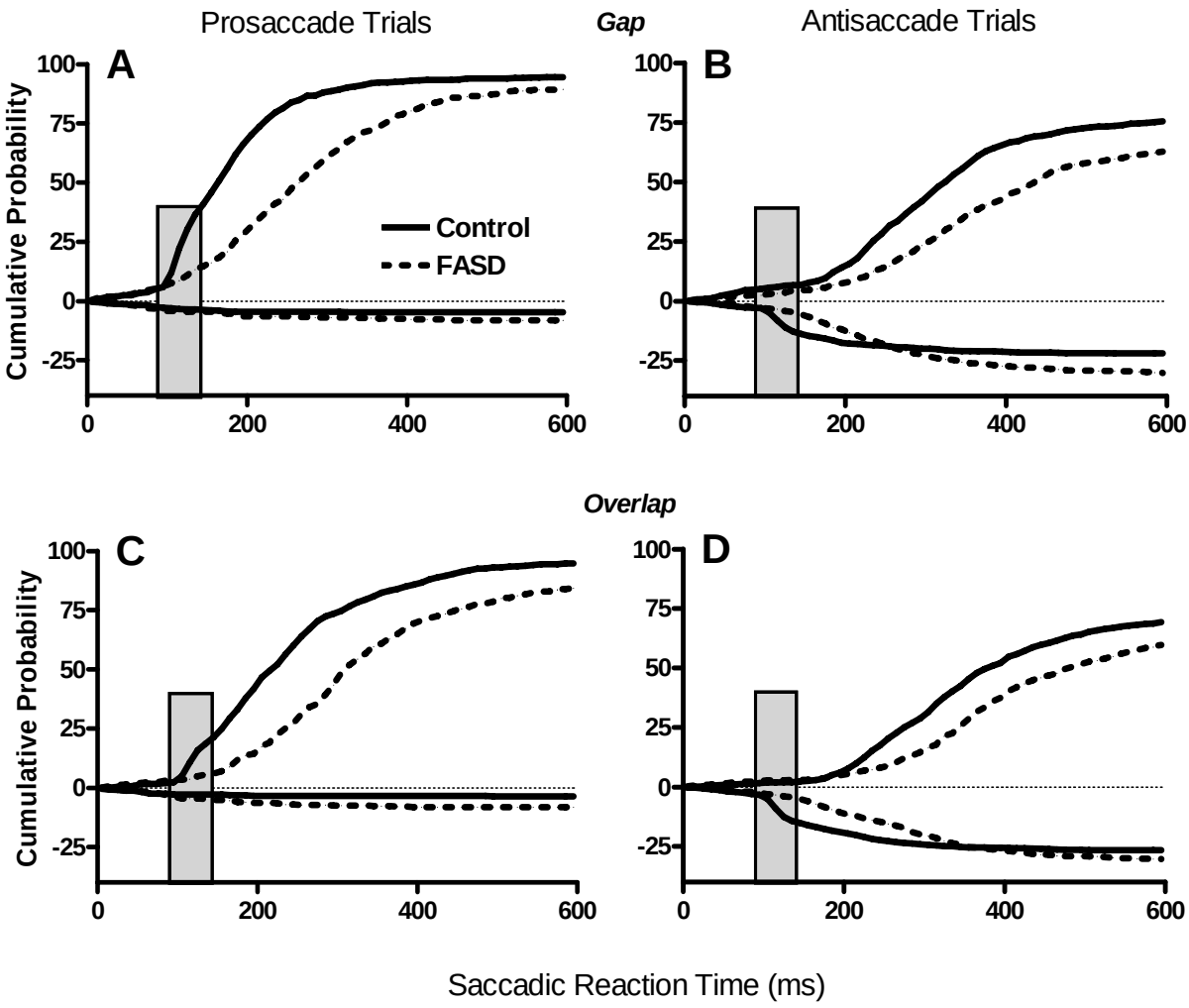

Fig. 2.1 Cumulative distribution of saccadic reaction times for correct responses (positive values on the ordinate) and direction errors (negative values on ordinate) for prosaccade (A, C) and antisaccade (B, D) trials in the gap (A, B) and overlap (C, D) conditions. Dashed traces, FASD data; solid traces, control data. The gray box highlights the express saccade epoch.

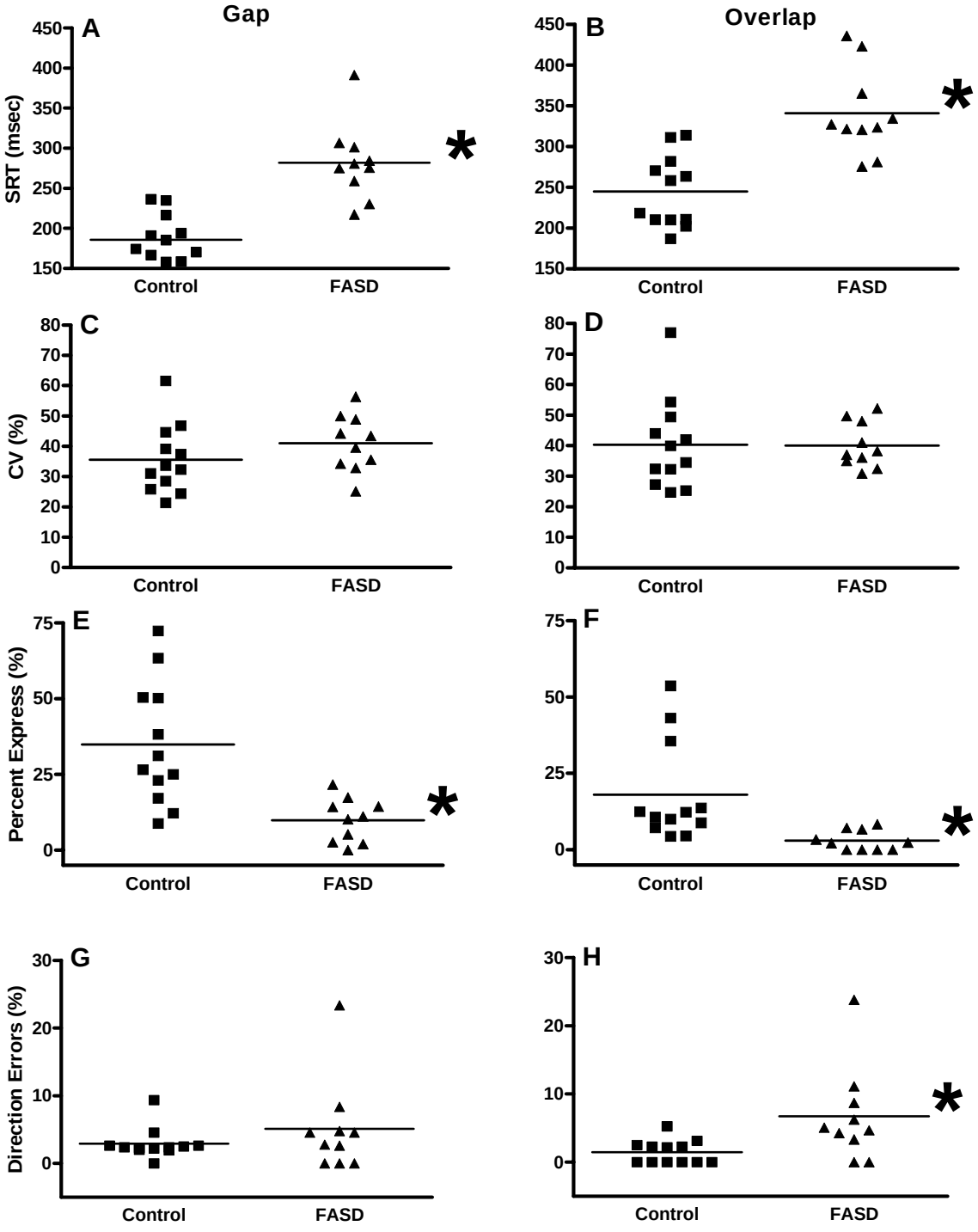

Fig. 2.2 Quantification of parameters in the gap (A,C,E,G) and overlap (B,D,F,H) conditions for the prosaccade task. *A* and *B*: mean SRTs for correct responses. *C* and *D*: coefficient of variation (SD of SRT/mean SRT x 100%). *E* and *F*: percentage of express saccades (SRT: 90-140 ms). *G* and *H*: percentage of direction errors. Values are presented as mean ± S.E.M. $^{*}p < 0.05$.

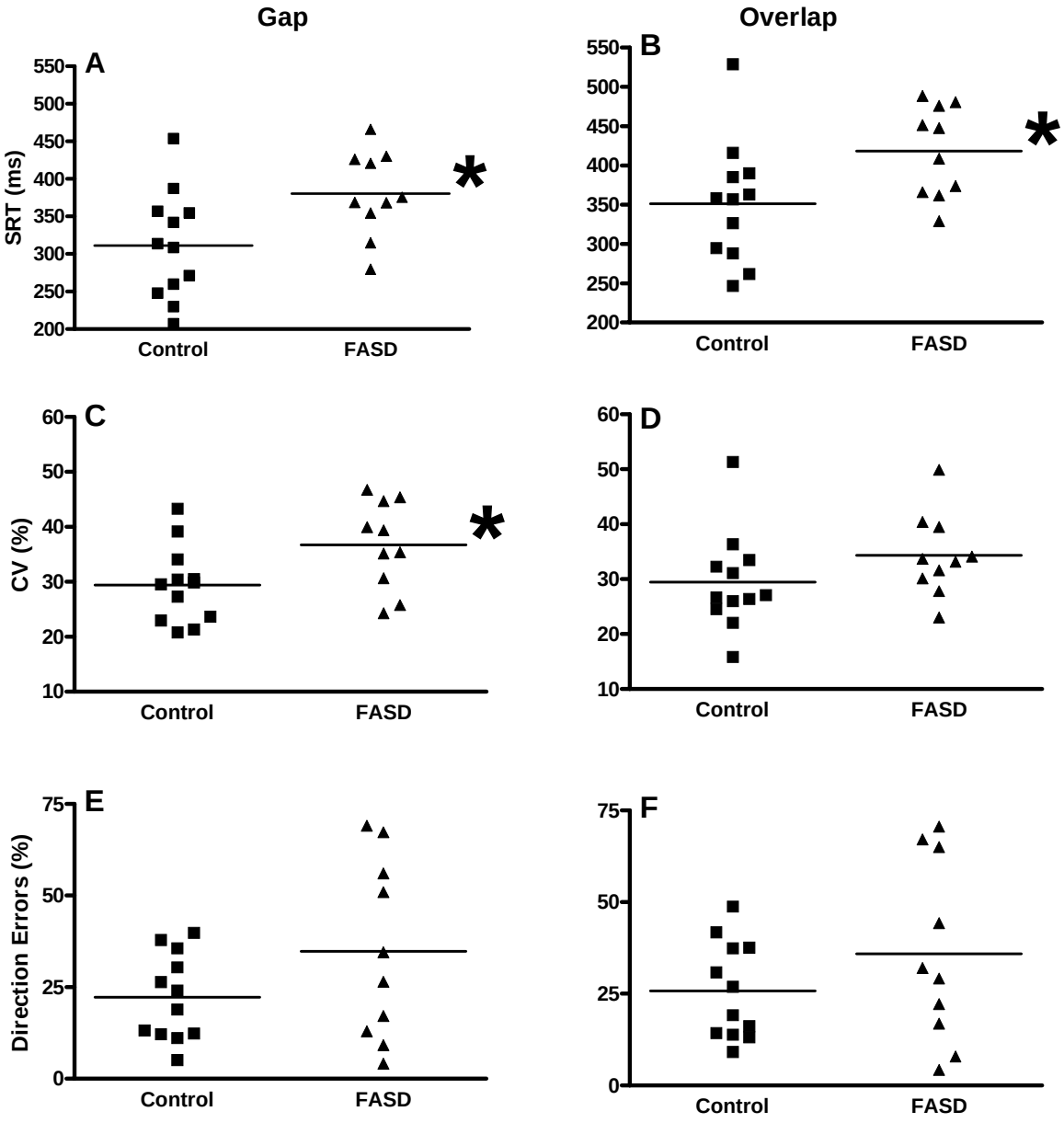

Fig. 2.3 Quantification of parameters in the gap (A,C,E) and overlap (B,D,F) conditions for the antisaccade task. *A* and *B*: mean SRTs for correct responses. *C* and *D*: coefficient of variation (SD of SRT/mean SRT x 100%). *E* and *F*: percentage of direction errors. Values are presented as mean ± S.E.M. $^{*}p < 0.05$.

prosaccade task (gap: $t_{(20)} = 5.8$, $p < 0.001$; overlap: $t_{(20)} = 4.6$, $p < 0.001$) and in the anti-saccade task (gap: $t_{(20)} = 2.5$, $p < 0.05$; overlap: $t_{(20)} = 2.3$, $p < 0.05$). The anti-effect (anti-saccade SRT – prosaccade SRT) for children with FASD was not significantly different from control children ($t_{(20)} = 0.95$, p = 0.35).

The gap-effect (overlap SRT – gap SRT) for pro-saccades ranged between 19 – 95 ms for control subjects and 28 – 177 ms for FASD children, and there was no significant difference between groups ($t_{(20)} = 0.02$, p = 0.98). Similarly, the gap effect for anti-saccades was also not significantly different between controls and FASD ($t_{(20)} = 0.16$, p = 0.87), and ranged between -2 – 75 ms and -55 – 100 ms for control and FASD children, respectively.

There was a significant interaction for group across direction and fixation condition ($F(1,20) = 5.81$, $p < 0.05$). In control subjects, SRT in the prosaccade task were faster for leftward saccades, whereas faster SRT in the antisaccade task were dependent on rightward saccades. In contrast, for the FASD group, faster SRT in the pro-saccade task were dependent on rightward saccades, and direction had no effect on SRT in anti-saccades.

2.3.2 Coefficient of Variation

The coefficient of variation (CV) normalizes for intrasubject variability in SRT. Only task was statistically significant for the CV, indicating increased variability in SRT for the antisaccade task compared to the prosaccade task ($F(1,20) = 8.5$, $p < 0.01$). There were no significant differences between group ($F(1,20) = 2.0$, p = 0.17), fixation condition ($F(1,20) = 0.9$, p = 0.77) or direction ($F(1,20) = 1.0$, p = 0.34).

Figures 2.2C and D illustrate the intra-subject variability in SRT for prosaccades, expressed as the CV. There was no significant difference between controls and FASD in CV for the gap ($t_{(20)} = 2.3$, p = 0.24) and overlap ($t_{(20)} = 0.04$, p = 0.97) conditions (Fig. 2.2C, D). Figures 2.3 C and D illustrate CV for control and FASD children in the anti-saccade task. CV was elevated for the FASD children in the gap condition ($t_{(20)} = 2.3$, $p < 0.05$], and the same trend was present in the overlap condition [$t_{(20)} = 1.4$, p = 0.18].

2.3.3 Express Saccades

The ANOVA of dependent measures revealed significant differences between group, task and fixation conditions with respect to express saccades. The control group generated a higher percentage of express saccades compared to the FASD children ($F(1,20) = 9.9$, $p < 0.01$). A higher percentage of express saccades were also generated in the prosaccade task compared to antisaccade task ($F(1,20) = 21.7$, $p < 0.001$) and for the gap condition compared to the overlap condition ($F(1,20) = 35.5$, $p < 0.001$. There were no significant differences in direction ($F(1,20) = 0.1$, p = 0.78).

There was an unexpected and significant decrease in the percentage of express saccades (Fig. 2.2E, F) by the FASD children in both the gap ($t_{(14)} = 4.0$, $p < 0.01$) and overlap ($t_{(12)} = 3.1$, $p < 0.01$) conditions. Express saccades are not generated in the anti-saccade paradigm, and saccades with SRT latencies comparable to the express epoch represent direction errors that are made toward the stimulus (see Fig. 2.1B, D).

There was an interaction between group and task ($F(1,20) = 13.1$, $p < 0.01$) such that increased percentage of express saccades depended on the gap condition in the control children, whereas this relationship was virtually absent in FASD children. There was also an interaction for group across task and fixation condition ($F(1,20) = 5.85$, $p <$

0.05), which revealed that increased express saccades in controls was dependent on pro-saccades in the gap condition. While there was no evidence, an interaction between fixation condition and group approached statistical significance ($F(1,20) = 3.80$, p = 0.07).

2.3.4 Direction Errors

There was a statistically significant increase in the percent of direction errors with respect to task ($F(1,20) = 36.1$, $p < 0.001$). Although there was no evidence for differences in group ($F(1,20) = 3.5$, p = 0.08) or fixation condition ($F(1,20)$, p = 0.07), both dependent measures approached statistical significance. There was no significant difference for direction ($F(1,20) = 0.17$, p = 0.68).

An unexpected finding was the increase in the percent of direction errors on pro-saccade trials in the overlap condition (Fig. 2.2 H) for the FASD group compared with controls ($t_{(10)} = 2.3$, $p < 0.05$), which did not reach significance in the gap condition ($t_{(20)} = 1.0$, p = 0.31) (Fig. 2.2 G). The percentage of direction errors in the antisaccade task is illustrated in Fig. 2.3, E and F. Although FASD children made more direction errors in the antisaccade task, this failed to reach statistical significance (gap: $t_{(12)} = 1.5$, p = 0.16; overlap: $t_{(13)} = 1.2$, p = 0.26).

2.3.5 Metrics

We also investigated saccade metrics for the saccades made in the prosaccade task (Table 2.2). There were no differences between control and FASD subjects for amplitude of the first saccade to stimulus ($F(1,20) = 1.69$, p = 0.21) or the number of saccades ($F(1,20) = 2.37$, p = 0.139). For saccades between 18-21° in amplitude, there was a strong trend toward an increase in duration ($F(1,20) = 3.76$, p = 0.07), and

Table 2.2 Saccade metrics from the prosaccade task.

	Amplitude (^{0})	Number of Saccades	Duration (ms)	Peak Velocity (^{0}/s)
Gap condition				
Control	19 ± 1	1.22 ± 0.05	75 ± 5	471 ± 25
FASD	19 ± 1	1.09 ± 0.06	88 ± 5	398 ± 27
Overlap condition				
Control	19 ± 1	1.15 ± 0.04	76 ± 5	445 ± 24
FASD	20 ± 1	1.07 ± 0.05	89 + 5	394 ± 26

reduction in saccade velocity ($F(1,20) = 3.08$, $p = 0.10$) for the FASD subjects compared to controls. It is expected that with a larger sample size these latter two measures would reach statistical significance.

2.4 Discussion

This study shows for the first time that saccadic eye movement experiments can be conducted in children with FASD, and suggests that these tests may provide a sensitive indicator of the brain injury associated with FASD. Significant differences between the performance of FASD subjects and control subjects were demonstrated. Children with FASD exhibited: 1) increased reaction times; 2) a decreased ability to trigger express saccades; 3) an increase in direction errors in the prosaccade task; and 4) no significant increase in direction errors in the antisaccade task. Therefore, we conclude that these data support our initial hypothesis that eye movement experiments can be used to assess executive function in children with FASD. These data are first discussed and related to other clinical groups with developmental disabilities. We then review saccade neural circuitry and speculate about FASD pathophysiology.

2.4.1 Eye Movement Abnormalities in Developmental Disorders

A frequent co-morbidity for individuals with FASD is attention-deficit hyperactivity disorder (ADHD) (Table 2.1); however, differences in neurocognitive and behavioral characteristics have been previously found (Coles et al., 1997). Several saccadic eye movement studies have been conducted in the ADHD population using pro- and antisaccade paradigms (Aman et al., 1998;Munoz et al., 2003;Klein et al., 2003;Hanisch et al., 2006;Cairney et al., 2001;Mostofsky et al., 2001). These studies have revealed contradictory results due largely to discrepancies in methodology. Two studies with rigorous procedural control and statistical analyses reveal that children with ADHD produce significantly more direction errors in the antisaccade task compared to controls (Munoz et al., 2003;Klein et al., 2003). However, these studies also present

contrasting results regarding the occurrence of express saccades. Munoz and colleagues (2003) described a trend toward ADHD children producing more express saccades compared to controls. In contrast, Klein and colleagues (2003) found that children with ADHD exhibited a reduced proportion of express saccades in the gap condition.

Although co-morbid ADHD existed in eight of the 10 cases of FASD, the emerging profile for saccadic eye movement abnormalities in FASD is markedly different from what is observed in children with a diagnosis of ADHD (Munoz et al., 2003;Klein et al., 2003). Children with FASD failed to generate express saccades, even among direction errors in the antisaccade task (see Fig. 2.1). In addition, they made more direction errors in the prosaccade task, while ADHD children made more direction errors in the ant-saccade task only. The increased percentage of direction errors in the pro-saccade task exhibited in FASD is highly unusual and not observed in ADHD. Direction errors in the antisaccade tasks tend to be initiated immediately following target appearance, within the express epoch for both control (Munoz et al., 1998) and ADHD (Munoz et al., 2003) subjects, while FASD subjects produce direction errors with much longer latencies (see Fig.2.1 B, D). Future studies will be important for exploring the potential use of saccadic eye movement experiments for contrasting FASD and ADHD. The marked differences we report in express saccade occurrence and pattern of direction errors provide important clues and definitely suggest dramatic differences in underlying pathophysiology.

Using the same tasks described here, LeVasseur and colleagues (2001) demonstrated that individuals with Tourette's syndrome (TS) have increased SRTs, no significant increase in direction errors in the antisaccade task, and a decrease in the

percentage of express saccades. Although this pattern of deficits resembles the data observed in FASD, children with FASD make more direction errors in the prosaccade task, while TS subjects do not. In addition, individuals with TS display no significant differences in saccadic velocity or duration, while the amplitude of the first saccade was smaller and more saccades were generated to move to the target. These observations contrast the trends observed in FASD subjects, in which a decrease in saccadic velocity and an increase in saccadic duration approached significance. These differences between TS and FASD suggest very different patterns of pathophysiology in these two disorders.

Of interest, our observations on FASD are not attributable to developmental delay. The normative data generated by Munoz and colleagues revealed that young children (ages 5-8) initiated more express saccades (Munoz et al., 1998) than what we observed in FASD. The increase in the occurrence of express saccades among young children suggests that they have poor control over visual fixation, leading to the generation of excessive automatic (reflexive) saccades. Fixation ability improves during normal maturation. The virtual absence of express saccades in the FASD children must therefore be due to a deficit that cannot be explained simply by a delay in normal development.

2.4.2 Neural Circuitry

The neural circuitry underlying saccadic eye movements has been well characterized (Munoz and Everling, 2004;Munoz and Schall, 2004;Pierrot-Deseilligny et al., 2004;Sparks, 2002;Scudder et al., 2002;Schall, 2004). The dorsolateral prefrontal cortex (dlPFC) is involved in executive function, spatial working memory, and importantly, the suppression of unwanted saccades; while the frontal eye fields (FEF)

play a crucial role in the execution of voluntary saccades. Punctate lesions to the dlPFC lead to increased direction errors in the antisaccade task (Gaymard et al., 1998;Guitton et al., 1985). Damage to the FEF is correlated with prolonged SRT (Rivaud et al., 1994). In the monkey, reversible inactivation of the FEF leads to increased SRT, decreased saccadic velocity and increased saccadic duration (Sommer and Tehovnik, 1997;Dias and Segraves, 1999). Reversible inactivation of the dlPFC, results in impairments in reflexive saccadic inhibition, leading to increased direction errors in the antisaccade task (Condy et al., 2006). These findings are similar to those obtained in our study, and suggest that dlPFC and FEF dysfunction may account, in part, for the oculomotor deficits observed in FASD. In FASD children, there appears to be global frontal lobe damage that may include the FEF and dlPFC, which would account for the increased SRT and excessive numbers of non-reflexive (longer-latency) direction errors in the anti-saccade task.

Damage to frontal lobes has been well documented in the FASD population (Wass et al., 2001;Sowell et al., 2002), and this has been correlated with deficits in impulsivity, response inhibition and judgement (Rasmussen, 2005). While our study failed to reveal deficits in impulsivity (increased reflexive direction errors in the anti-saccade task), it was apparent that children with FASD executed more disorganized responses that reflected increase direction errors in both the pro- and antisaccade tasks. Additionally, their inability to turn off the fixation mechanism in advance precluded their capacity to generate express saccades. These observations suggest that FASD children have difficulty integrating multiple instructions into a correct sequence of actions, which

is consistent with the known deficits in planning ability and/or response inhibition that are associated with FASD.

The relationship between dlPFC and express saccades has been previously investigated using single pulse transcranial magnetic stimulation (TMS) and saccadic eye movement experiments (Muri et al., 1999). The reduction in the SRT following TMS of the dlPFC was attributed to an increase in the percentage of express saccades. The authors suggested that this effect was mediated by either direct activation of the superior colliculus (Leichnetz, 1981) or disinhibition of the superior colliculus via the basal ganglia (Hikosaka et al., 2000). Thus, alterations in the pattern of signalling from dlPFC to superior colliculus may result in deficient express saccade generation. Deficits in FEF function can lead to increased latencies, which could further contribute to the increase in prosaccade duration and decrease in prosaccade velocity. Taken together, these results suggest that global deficits among regions of the frontal cortex lead to a specific pattern of eye movement behavior that is unique to the FASD population consisting of increased SRT and decreased percentage of express saccades. One plausible explanation for these observed deficits is increased inhibition within frontal cortex. Studies conducted in experimental animal models have shown that chronic prenatal ethanol exposure induces an upregulation in the expression of $GABA_A$ receptors in the cerebral cortex of postnatal offspring (Bailey et al., 1999;Bailey et al., 2001), which would be expected to increase inhibitory tone.

2.4.3 The Accumulator Model

Several models have been proposed to explain the variability of reaction time (Luce, 1986;Munoz and Schall, 2004;Ratcliff, 2006;Nazir and Jacobs, 1991;Trappenberg

et al., 2001). The accumulator model is useful for interpreting the neurophysiological and behavioral data related to the initiation of saccades in clinical disorders (Munoz et al., 2007). This model is based on the supposition that to initiate movement, neural activity must grow to exceed a given threshold (Fig. 2.4). In the brain, this function may be represented by the pre-saccadic activity of saccade neurons in the FEF and superior colliculus (Munoz and Schall, 2004). These neurons can be activated in advance of target presentation, as well as activated directly by target presentation in a neuron's response field (post-target activation). Three sources have been identified as contributors to variability in reaction time: baseline, threshold, and rate of rise (from baseline to threshold). Thus, pre-target (baseline) and post-target (rate of rise) information processing can alter the accumulation of activity towards threshold to initiate action. In Fig. 2.4, the vertical gray box represents the time of the visual response on saccade neurons contralateral to the side of the target. Pre-target activity (to the left of the target appearance in Fig. 2.4) is influenced by fixation disengagement and target predictability (Munoz et al., 2000).

The accumulator model has been used to account for deficits in ADHD, TS and Parkinson's disease (Munoz et al., 2007). Mean SRT was elevated in ADHD, more direction errors were generated in the antisaccade task compared to controls, and there was a trend toward more express saccades and excessive variability in SRT. These observations suggest poor control over pre-target activity. Excessive pre-target activity can combine with the phasic visual response on the contralateral side to trigger express saccades in the prosaccade task (Fig. 2.4A, black dashed traces) and reflexive direction errors in the antisaccade task (Fig. 2.4B, black dotted trace). ADHD behavior can be

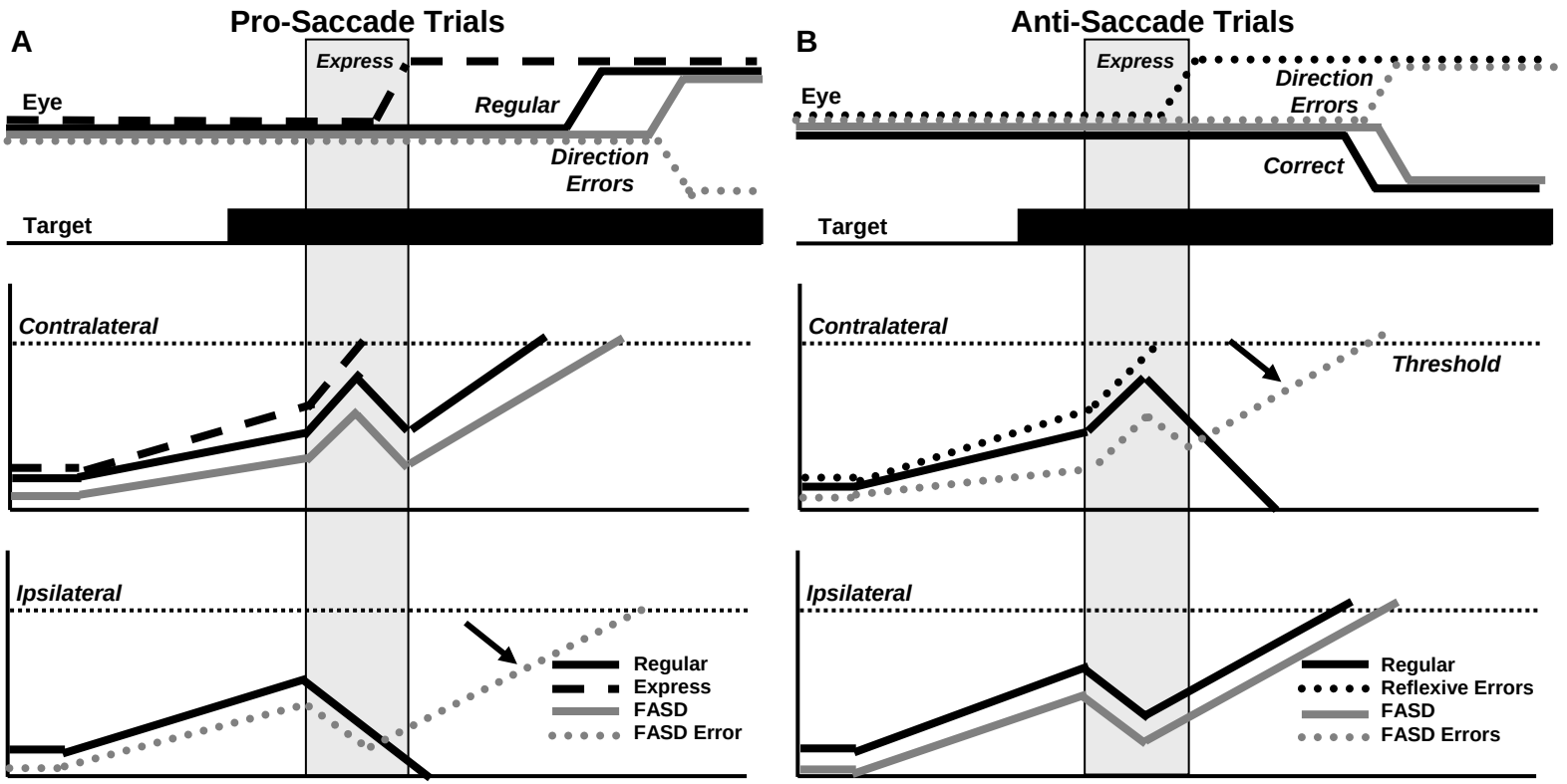

Fig. 2.4 Accumulator model to describe eye movement abnormalities in FASD. Neural activity along the y-axis must grow to reach a given threshold to trigger a saccade. (A) In the pro-saccade task, with sufficient pre-target activity, the visual response can lead to the immediate threshold crossing and trigger an express saccade (black dashed trace). Reduced pre-target activity leads to the initiation of regular-latency saccades (black solid trace). In FASD, pre-target activity is reduced, leading to delayed threshold crossing (grey solid trace). In addition, activity is allowed to increase toward threshold on the ipsilateral side of the brain leading to highly unusual direction errors in the pro-saccade task (dotted grey trace). (B) To initiate a correct anti-saccade, activity must cross threshold on the ipsilateral side of the brain. If pre-target activity is too high, the post-target visual response can contribute to drive the contralateral accumulator over threshold triggering a reflexive direction error (black dotted trace). In FASD, reflexive direction errors are not triggered but longer latency errors can occur (grey dotted trace).

accounted for by deficits in managing pre-target excitability. Post-target factors appeared somewhat normal in ADHD (Munoz et al., 2007). LeVasseur and colleagues (2001) suggested that the increased SRT and reduced occurrence of express saccades in TS may be due to a reduction in pre-target activity leading to delayed threshold crossing (similar to grey traces in Fig. 2.4).

Recall that children with FASD exhibit increased SRT, a decreased ability to trigger express saccades, increased direction errors in the prosaccade task and no significant increase in direction error in the antisaccade task. We speculate that FASD children have reduced excitability (reduced pre-target activity) in the saccade generating circuit (Fig. 2.4, grey traces below black traces). As a consequence, in the prosaccade task, an express saccade is never triggered, while in the antisaccade task, no direction errors are triggered at express saccade latency. Instead, the reduced pre-target baseline leads to prolonged reaction times. A striking difference between FASD and both TS and ADHD, was the increase in the occurrence of direction errors in the prosaccade task. Because of poor inhibitory control in FASD, inappropriate activity is allowed to increase toward threshold on the ipsilateral side of the brain (Fig. 2.4A, see arrow and dotted grey trace) leading to some direction errors in the prosaccade task, which is highly abnormal.

To initiate a correct antisaccade (Fig. 2.4B), activity must cross threshold on the ipsilateral side of the brain. If pre-target activity is too high, the post-target visual response on the contralateral side can contribute to drive the system over threshold triggering a reflexive direction error (Fig. 2.4B, dotted black trace). Because of excessive inhibition in FASD, reflexive direction errors are not triggered, but longer latency direction errors can occur (Fig. 2.4B, see arrow and dotted grey trace).

Although, the specific mechanisms remain to be determined, our suggestions of reduced pre-target activity and accumulation of activity on the wrong side (i.e., ipsilateral side for prosaccades; contralateral side for antisaccades; see arrows in Fig. 2.4) is unique for FASD and is dramatically different to what is proposed for ADHD subjects(Munoz et al., 2007). As discussed above, this may be the result of an upregulation in the expression of $GABA_A$ receptors (Bailey et al., 1999;Bailey et al., 2001), which would lead to increased inhibition. Such a mechanism could lead to oculomotor abnormalities, and explain the reduction in baseline activity among saccade neurons (Fig. 2.4). Although FASD subjects do not appear to be impaired in their ability to inhibit automatic visually triggered saccades in the antisaccade task, it is clear that they do have deficits is suppressing activity calling for inappropriate saccades (direction errors) at longer latency. The exact mechanism by which increased inhibition translates into these abnormal processes remains to be fully determined.

2.4.4 Study Limitations

As the sample size was small, it was not possible to confirm with any certainty the contribution that medication and/or co-morbidity may have had to the findings presented here. It appears from the scatter plots that the effects reported in this study were not driven by lone subjects, who were considered outliers (Figs. 2.2 and 2.3). Still, the importance of these possible confounders is noted and will be more fully investigated in future larger scale studies.

2.4.5 Conclusion

To the best of our knowledge, this study is the first of its kind to evaluate control of saccadic eye movements in children with FASD. A preliminary report of eye

movement recording in young adults with prenatal alcohol exposure reported similar effects (Willford et al., 2005). Results from the larger scale study will more fully characterize the sensitivity and specificity of these deficits in the FASD population. The results from this preliminary study suggest that measuring saccadic eye movement behavior is a promising research and diagnostic tool for evaluating the brain injury resulting from prenatal ethanol exposure.

Chapter 3 EXECUTIVE FUNCTION DEFICITS IN CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDERS ASSESSED USING A MOBILE EYE TRACKING LABORATORY

3.1 Introduction

Adverse outcomes occurring in offspring as a consequence of prenatal exposure to alcohol are documented (McGee and Riley, 2006;Kodituwakku, 2007). Fetal alcohol spectrum disorders (FASD) is the umbrella term used to represent the full range of teratogenic effects attributed to gestational alcohol exposure, including fetal alcohol syndrome (FAS) (Koren et al., 2003). An FAS diagnosis requires the presence of pre- and postnatal growth restriction, craniofacial dysmorphology and central nervous system dysfunction (Chudley et al., 2005;Clarren and Smith, 1978). In the absence of one or more of these features, individuals may receive a diagnosis of partial FAS (pFAS) or alcohol-related neurodevelopmental disorder (ARND).

Individuals with FASD may present with a range of impairments in executive function (Funahashi, 2001;Lezak, 1995) that include deficits in spatial working memory, planning, response inhibition, abstract thinking and the ability to shift attention (Kodituwakku, 2007;Rasmussen, 2005). Impairments in executive function and social skills reported by parents and teachers demonstrate that pervasive deficits impact on social behaviors across multiple settings (Schonfeld et al., 2006).

Measurement of eye movement control is a powerful tool for assessing executive function (Munoz and Everling, 2004). Extensive literature based on neurophysiological, anatomical, imaging and lesion studies has contributed to our understanding of the neural circuits controlling saccadic eye movements (Leigh and Zee, 1999;Sweeney et al.,

2007;Heide and Kompf, 1998;Pierrot-Deseilligny et al., 2004), and paradigms have been used extensively in basic and clinical research (Ramat et al., 2007;Munoz et al., 2007).

In this study, subjects were required to look either toward (prosaccade) or away from (antisaccade) a peripheral target that appeared on a computer monitor. Prosaccades can be triggered automatically by visual inputs to the saccade generating circuit from the visual and posterior parietal cortices (Munoz and Everling, 2004). Antisaccades require additional steps of processing: suppression of the automatic prosaccade; and initiation of the voluntary antisaccade. Successful antisaccade performance relies on circuitry that includes higher brain centers such as the frontal cortex and basal ganglia (Munoz and Everling, 2004). Deficits in parietal and frontal cortices, and basal ganglia have been previously reported in FASD (McGee and Riley, 2006) making saccade tasks an appropriate tool for assessing executive function.

In a previous report (Green et al., 2007c), we described eye movement abnormalities in a small cohort of children with FASD (Chapter 2). However, the sample size was too small to determine the effects of development or diagnosis within the FASD spectrum on oculomotor control. To address these important questions, we developed a mobile laboratory that facilitated eye movement testing in different communities across Canada. We hypothesized that children with FASD display a developmental delay in eye movement control, such that younger children exhibit relatively greater deficits compared with older children. Moreover, we predicted that differences in the magnitude of deficits in oculomotor control exist among the diagnostic subgroups, such that the children with FAS demonstrate the most profound deficits.

Preliminary versions of these data have been presented in abstract form (Green et al., 2007b;Green et al., 2007a).

3.2 Materials and Methods

3.2.1 Participants

All experimental procedures were reviewed and approved by the Human Research Ethics Boards of Queen's University, the Children's Hospital of Eastern Ontario (Ottawa subjects), and the University of Alberta (Edmonton subjects). Children with FASD were recruited from 8 different communities across Ontario and one community from Alberta (see Table 3.1). A total of 189 subjects were recruited into the study: 92 (40 males, 52 females; 11.2 ± 0.2 years of age, range 8-15 years) were control children (non-FASD) and 97 were grouped as FASD. Of the 97 subjects included in the FASD group, 89 (44 males, 45 females; 10.7 ± 0.2 years of age, range 8-15 years) had a diagnosis within the FASD spectrum (FAS, pFAS, ARND), while 8 were suspected and/or exposed, but had yet to receive a definitive diagnosis. The children with FASD were previously assessed at local diagnostic clinics in accordance with the Canadian Diagnostic guidelines (Chudley et al., 2005). Data sets from one control subject and one subject with FASD were lost due to equipment problems. All data included in the analysis for the FASD group were obtained from the 88 children who had received a diagnosis within the spectrum and 91 controls. For the purpose of analysis based on age, children were placed in one of three separate age bins: 8-10 years, 11-12 years and 13-15 years.

Of the 89 children with FASD, 60 were medicated for behavioral symptoms relating to their co-morbidities (i.e., stimulant and antipsychotic agents). On the test day, primary caregivers were asked to withhold medication until the testing was completed. Of the 60 children taking medications, 8 were tested on medication (87% compliance

Table 3.1: Test locations and subject breakdown.

TEST SITE	FASD (N)	CONTROL (N)
Cobourg, ON	0	12
Durham, ON	11	3
Edmonton, AB	17	2
Kenora, ON	11	0
Kingston, ON	11	46
Ottawa, ON	9	10
Peterborough, ON	6	5
Sioux Lookout, ON	15	6
Toronto, ON	9	8
Total:	**89**	**92**

with the off medication request). For the remaining 52 children, their last daily dose was administered a minimum of 12 hours prior to testing.

All control subjects had no known neurological, psychiatric or visual disorders, other than requiring corrective lenses. Primary caregivers were informed of the nature of the study and provided written consent on behalf of the participants. All subjects completed one, 1-hour eye movement session. Each subject received $10 and a small gift for participating in the study.

3.2.2 Saccade Task

All participants performed a blocked design saccade task (Fig. 1.1), consisting of 2 blocks of prosaccade and 2 blocks of antisaccade trials, each consisting of 80 trials (320 trials total). Subjects received breaks when needed, and refreshments were provided upon completion of the task. Participants were seated comfortably in a darkened room, facing the center of a laptop screen located 46 cm away. Task presentation on the laptop screen was produced using E-prime software. A red spot with a luminosity of ~12.5 cd/m^2; and x=0.57 and y=0.32 coordinates in CIE space (relative to the background lumination of ~ 1.0 cd/m^2; and x=0.34 and y=0.34 coordinates in CIE space) was positioned at the center of the screen and served as the initial fixation point (FP). Red target (T) spots were positioned on the screen at 15° to the right or left of the central FP. The screen was diffusely illuminated between trials to avoid dark adaptation. Each trial began with a 250-ms period of darkness. The FP appeared for 1,000 ms and then one of two conditions occurred (Fig. 1.1). In the gap condition, the FP was extinguished and, after a period of 200 ms, the target appeared in the right or left visual field. In the overlap condition, the FP remained illuminated while the target appeared.

In the block of prosaccade trials, participants were instructed to look toward the target as soon as it appeared. In the block of antisaccade trials, participants were instructed to look away from the target to the opposite side. The target remained illuminated for 1,000 ms, after which all visual stimuli disappeared and the background illumination reappeared, indicating the end of that trial. Target location (right or left) and fixation condition (gap or overlap) were pseudo-randomly interleaved throughout each block of trials. Subjects were asked to repeat and demonstrate the instructions to the experimenter to ensure that they understood the task before the onset of data collection.

3.2.3 Recording and Analysis of Eye Movement

The video-based infrared eye-tracker (ISCAN Inc., Burlington, MA) was adapted for use as a mobile laboratory, and transported to each test center. Eye position was measured using a head-mounted camera that was connected to a data acquisition computer. The video-based infrared eye-tracker, tracked the pupil movement, extracting measures of eye position and pupil size at a sampling rate of 240 Hz. Only left eye position was digitized. Saccades were detected offline at 3 standard deviations above the background and must have lasted longer than 5 sample points (Matlab, custom software).

Saccadic reaction time (SRT) was defined as the time from target appearance to initiation of the first saccade that exceeded 30°/s. Saccades were scored as correct if the first movement after target appearance was $> 5^{\circ}$ in amplitude and in the correct direction (i.e., toward the target for prosaccades; away from the target for antisaccades). Saccades were scored as incorrect if the first saccade after the appearance of the target was in the wrong direction relative to the instruction (i.e., away from the target in the prosaccade, toward the target in the antisaccade). All saccade marks and direction errors were verified

off-line. The mean SRT in the prosaccade and antisaccade task was computed from all correct trials with reaction latencies between 90 and 1000 ms to eliminate short-latency anticipatory saccades (Munoz et al., 1998). In addition, we measured express saccades (latency: 90-140 ms), which are the shortest latency visually triggered saccades (Dorris et al., 1997;Fischer et al., 1993); and this express epoch was confirmed for the mobile laboratory. There was some variability in the experimental conditions across multiple test sites. Most notable was the amount of ambient light in which the test sessions were conducted, and we attempted to control for this variability by covering external light sources (i.e., windows) with curtains or sheets. However, target luminosity changed very little regardless of these differences in background ambient light. We also maintained similar set-up protocols to ensure that equipment and experimenter/subject space was consistent for each test site.

The following parameters were computed for each condition (gap, overlap): the mean SRT for correct trials, the coefficient of variation (CV) of SRT for correct trials [(CV = standard deviation/mean) x 100], the percentage of express saccades, and the percentage of direction errors.

3.2.4 Inclusion/Exclusion criteria

In order to determine inclusion and exclusion criteria, SRT histograms were prepared for each subject for each experimental task (pro, anti) and condition (gap, overlap). From these figures, subjects were placed in bins according to their performance. For example, Selection A included all subjects who could perform saccades under each task and condition, while Selection E reflected those subjects who could only perform prosaccades. This approach provided a means for excluding subjects

who could not perform certain tasks or in situations where only a minimum number of trials were completed under a given condition (Table 3.2). Univariate data analyses were conducted for each outcome measure for each task (pro- and antisaccade) in each condition (overlap and gap), which included only the data sets from those subjects who were successful in performing the given task in the specified condition. Subsequently, analysis including both tasks and both conditions demonstrated that the statistical comparisons obtained from the complete data set were not different from the individual univariate analyses. Therefore, statistically significant outcome measures from the complete data set were a true representation of the study population.

3.2.5 Data Analysis

The two experiments (prosaccade and antisaccade) contained one within-subject factor: fixation state (gap vs overlap); and two between-group factors: clinical pathology (FASD vs control) and age (bins: 8-10 years, 11-12 years and 13-15 years). Unless specified otherwise, all dependent measures (SRT, CV, express saccades, direction errors) were analyzed using ANOVA with α set at 0.05. Difference scores (i.e., anti-effect and gap-effect) were analyzed using two-tailed, unpaired Student's *t*-tests corrected with Welch's approximation when the assumption for homogeneity of variance was not met. The effect of diagnosis (ARND, pFAS and FAS) was also determined by matching each subject in the FASD group (as close as possible) to a control subject by age and sex. FASD and control subjects once subdivided were analyzed by univariate analyses to test for differences between the diagnostic groups; and t-tests were conducted to contrast the pairs. We focus on descriptions of the relevant statistical parameters for comparisons and interactions that occurred between the control and FASD groups.

Table 3.2. Subject performance breakdown by task and condition for children with FASD and controls.

Selection	**A**	**B**	**C**	**D**	**E**	**F**	**G**	
	PG; PO AG; AO	**PG; PO AO**	**PG; PO AG**	**PG AO**	**PG; PO**	**PG**	**None**	**Total****
FASD	53	7	5	2	16	2	3*	88
Control	82	6	0	0	3	0	0	91

P=prosaccade; A=antisaccade; G=gap condition; O=overlap condition
*Two subjects did not attempt the antisaccade trials.
**One subject with FASD and one control subject data sets were lost due to equipment problems.

3.3 Results

3.3.1 Saccadic Reaction Time

Figure 3.1 depicts the cumulative distribution of SRT for correct responses (positive values) and direction errors (negative values) in all experimental conditions for control children and those diagnosed with ARND, pFAS or FAS. Children with FASD had longer SRTs compared with controls [$F(1,165) = 18.6$, $p < 0.001$]. Consistent with previous studies (Munoz et al., 1998;Dafoe et al., 2007), the mean SRT was increased for antisaccades compared with prosaccades [$F(1,165) = 649.0$, $p < 0.001$], and in the overlap condition compared with the gap condition [$F(1,165) = 531.5$, $p < 0.001$] for all groups.

The *anti-effect* (anti SRT – pro SRT) provides a measure of the difference in reaction times for anti- and prosaccades, thus illustrating differences in the voluntary and automatic mechanisms. The anti-effect for children with FASD was not significantly different from control children in the overlap or gap conditions ($p > 0.05$). The mean anti-effect was 100 ± 7 ms and 119 ± 7 ms for children with FASD in the overlap and gap condition, respectively; and 94 ± 5 ms and 109 ± 4 ms for controls, respectively.

The *gap-effect* (overlap SRT-gap SRT) provides a measure of the difference between fixation conditions, and serves to illustrate whether there are deficits in the processes of disengagement from fixation. The mean gap-effect for prosaccades was 71 ± 4 ms for children with FASD and 75 ± 3 ms for control subjects, and there was no significant difference between groups ($p > 0.05$). Similarly, the gap effect for antisaccades was also not significantly different between the two groups ($p > 0.05$), and the mean was 51 ± 7 ms and 60 ± 2 ms for FASD and control subjects, respectively.

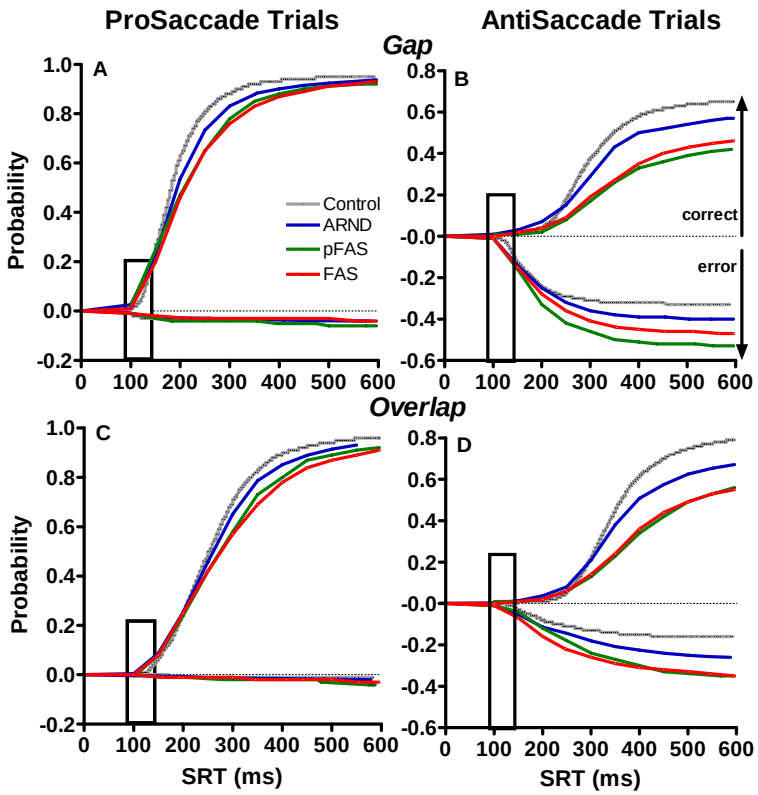

Figure 3.1. Cumulative distribution of saccadic reaction times for correct responses (positive values on the ordinate) and direction errors (negative values on the ordinate) for prosaccade (A,C) and antisaccade (B,D) trials in the gap (A,B) and overlap (C,D) conditions. Blue solid traces, alcohol-related neurodevelopmental disorder (ARND) data; green solid traces, partial fetal alcohol syndrome (pFAS) data; red solid traces, fetal alcohol syndrome (FAS) data; gray solid traces, control data. The open box highlights the express saccade epoch (90-140 ms).

After pairing each child within the diagnostic subgroup with the appropriate control, unpaired t-tests were conducted. In the prosaccade task, complete data sets were obtained from 42 children with ARND, 18 with pFAS and 25 with FAS; and in the antisaccade task, there were 41 children with ARND, 18 with pFAS and 24 with FAS. In comparison to their matched control, children with ARND had greater prosaccade SRTs in the gap condition $t_{(68)} = 3.3$, $p < 0.01$, but not in the overlap condition ($p > 0.05$); and greater antisaccade SRTs in the overlap condition $t_{(80)} = 2.0$, $p < 0.05$, which approached significance in the gap condition ($p = 0.08$). Children with pFAS were not significantly different from their matched control with respect to prosaccade SRTs in the overlap condition ($p > 0.05$), although scores approached significance in the gap condition ($p = 0.06$); and antisaccade SRTs were significantly greater than the matched controls in the gap condition $t_{(34)} = 2.9$, $p < 0.01$, and approached significance in the overlap condition ($p=0.06$). Compared to their matched controls, children with FAS demonstrated greater prosaccade SRTs in the gap $t_{(36)} = 3.1$, $p < 0.01$ and overlap $t_{(37)} = 2.3$, $p < 0.05$ conditions; and similarly for antisaccade SRTs in the gap $t_{(35)} = 3.3$, $p < 0.01$ and overlap $t_{(47)} = 2.2$, $p < 0.05$ conditions (Figs. 3.2 and 3.3).

We also were interested in determining whether there were significant differences between children with ARND, pFAS and FAS across the different outcome measures. There were no significant differences among the diagnostic subgroups for prosaccade SRTs in the gap or overlap conditions (Fig. 3.2A and B); nor was there a difference for antisaccade SRTs in the overlap condition (Fig. 3.3B). However, there was a significant difference for antisaccade SRT in the gap condition $F(2,80) = 5.4$, $p < 0.01$, such that

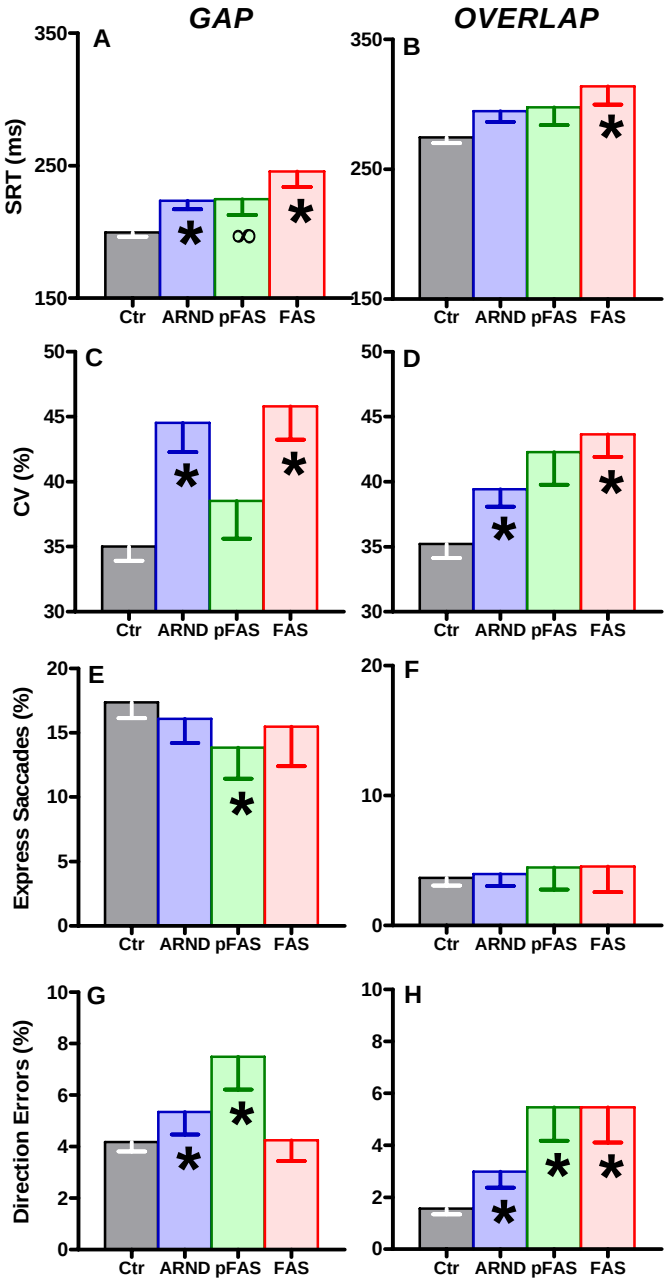

Figure 3.2. Quantification of parameters for the prosaccade task in the gap (A,C,E,G) and overlap (B,D,F,H) conditions. (A and B): mean saccadic reaction times (SRT) for correct responses. (C and D): coefficient of variation in SRT (SD of SRT/mean SRT x 100%). (E and F): percentage of express saccades (SRT: 90-140 ms). (G and H): percentage of direction errors. Grey, control (Ctr) data (subgroups collapsed); blue, alcohol-related neurodevelopmental disorder (ARND) data; green, partial fetal alcohol syndrome (pFAS) data; red, fetal alcohol syndrome (FAS) data. *$p \leq 0.05$ compared with matched-control subjects; $^{\infty}0.05 < p < 0.1$ compared with matched-control subjects.

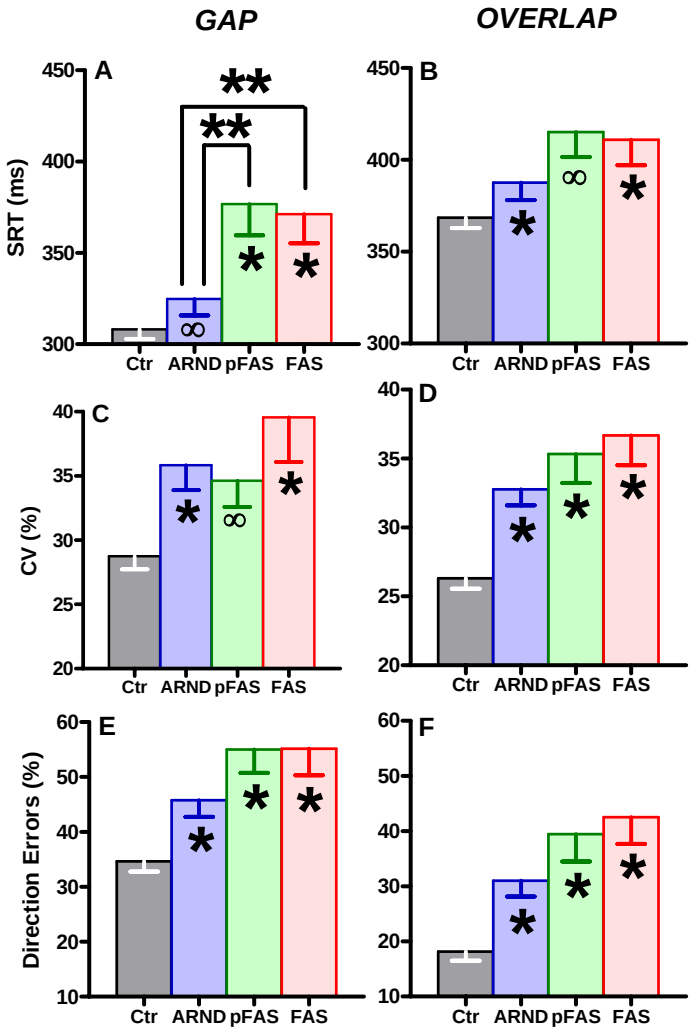

Figure 3.3. Quantification of parameters for the antisaccade task in the gap (A,C,E) and overlap (B,D,F) conditions. (A and B): mean saccadic reaction times (SRT) for correct responses. (C and D): coefficient of variation in SRT (SD of SRT/mean SRT x 100%). (E and F): percentage of direction errors. Grey, control (Ctr) data (subgroups collapsed); blue, alcohol-related neurodevelopmental disorder (ARND) data; green, partial fetal alcohol syndrome (pFAS) data; red, fetal alcohol syndrome (FAS) data. *$p \leq 0.05$ compared with matched-control subjects; **$p \leq 0.05$ statistical significance between the FASD diagnostic subgroups (A); $^{\infty}0.05 < p < 0.1$ compared with matched control subjects.

children with pFAS and FAS had greater SRT than children with ARND ($p < 0.05$) (Fig. 3.3A). This difference in SRT performance in the gap condition was also reflected in the anti-effect. There was a significant difference in the anti-effect only in the gap condition [antisaccade SRT – prosaccade SRT] ($F(2,80) = 4.2$, $p < 0.05$): children with pFAS had a greater anti-effect compared to the children with ARND ($p < 0.05$). There was no statistically significant difference between children with FAS and ARND ($p > 0.05$), or between children with FAS and pFAS ($p > 0.05$).

3.3.2 Coefficient of Variation in SRT

The CV expresses the intra-subject variability in SRT. Children with FASD demonstrated greater variability compared to controls [$F(1,165) = 32.0$, $p < 0.001$]. This difference in SRT variability among children in the FASD group is likely due to increased heterogeneity in task performance resulting from differing degrees of brain injury, and subsequent dysfunction, following prenatal alcohol exposure.

In comparison to their matched control, children with ARND had greater prosaccade CV in the gap $t_{(82)} = 3.1$, $p < 0.01$ and overlap $t_{(82)} = 3.0$, $p < 0.01$ conditions; and greater CV for antisaccades in the gap $t_{(80)} = 3.1$, $p < 0.01$ and overlap $t_{(80)} = 4.3$, $p < 0.001$ conditions. Children with pFAS were not significantly different from their matched control with respect to prosaccade CV in either the gap or overlap conditions ($p > 0.05$); however, CV for antisaccades was significantly greater in the overlap condition $t_{(34)} = 3.0$, $p < 0.01$ and approached significance in the gap condition ($p = 0.06$). Compared to their matched controls, children with FAS demonstrated greater prosaccade CV in the gap $t_{(48)} = 2.7$, $p < 0.05$ and overlap $t_{(48)} = 2.1$, $p < 0.05$ conditions; and

similarly CV for antisaccades was also greater in the gap $t_{(47)} = 2.3$, $p < 0.05$ and overlap $t_{(35)} = 3.6$, $p < 0.01$ conditions.

Among the diagnostic subgroups, there were no significant differences in CV for prosaccades (Figs. 3.2C and D) or antisaccades (Figs. 3.3C and D) in the gap or overlap conditions ($p > 0.05$).

3.3.3 Express Saccades

In contrast to our previous findings (Green et al., 2007c) where children with FASD generated significantly fewer express saccades, there was no effect of group on the proportion of express saccades in either the gap or overlap conditions ($p > 0.05$).

In comparison to their matched control, children with ARND demonstrated no significant differences in the percentage of express saccades generated in either the gap or overlap conditions ($p > 0.05$). Children with pFAS generated significantly fewer express saccades in the gap condition $t_{(34)} = 2.0$, $p = 0.05$ compared to their matched controls, but no significant difference in the overlap condition ($p > 0.05$). There was no significant difference in the percentage of express saccades generated by children with FAS compared to their matched controls in either the gap or overlap conditions ($p > 0.05$).

Among the diagnostic subgroups, there were no significant differences for the percentage of express saccades in the prosaccade task in the gap or overlap conditions (Figs. 3.2E and F) ($p > 0.05$).

3.3.4 Direction Errors

Consistent with previous literature (Munoz et al., 1998), there was a significant increase in the percentage of direction errors in the antisaccade task [$F(1,165) = 461.8$, $p < 0.001$] compared with the prosaccade task, and in the gap condition [$F(1,165) =$

260.9, $p < 0.001$] compared with the overlap condition. Children with FASD made more direction errors compared to controls [$F(1,165) = 30.5$, $p < 0.001$].

In comparison to their matched control, children with ARND generated more direction errors for prosaccades in the gap $t_{(59)} = 2.0$, $p = 0.05$ and overlap $t_{(55)} = 2.4$, $p < 0.05$ conditions; and more directions errors for antisaccades in the gap $t_{(80)} = 4.2$, $p < 0.001$ and overlap $t_{(69)} = 5.0$, $p < 0.001$ conditions. Children with pFAS were significantly different from their matched control with respect to direction errors for prosaccades in the gap $t_{(28)} = 2.1$, $p < 0.05$ and overlap $t_{(23)} = 2.2$, $p < 0.05$ conditions; and for antisaccades in the gap $t_{(34)} = 2.0$, $p = 0.05$ and overlap $t_{(34)} = 2.0$, $p < 0.05$ conditions. Compared to their matched controls, children with FAS demonstrated greater prosaccade direction errors in the overlap $t_{(30)} = 2.8$, $p < 0.05$, but not gap ($p > 0.05$) condition; and increased direction errors for antisaccades in the gap $t_{(47)} = 3.1$, $p < 0.01$ and overlap $t_{(33)} = 4.2$, $p < 0.001$ conditions.

Among the diagnostic subgroups, there were no significant differences in the percentage of direction errors for prosaccades in the gap or overlap conditions (Fig. 3.2G and H) ($p > 0.05$); nor were there differences for antisaccades in the gap condition (Fig. 3.3E) ($p > 0.05$). A trend was apparent for direction errors in the antisaccade task in the overlap condition ($F(2,80) = 2.6$, $p = 0.08$) (Fig. 3.3F), such that the children with ARND tended to make fewer direction errors compared to FAS ($p = 0.1$). The same trend was not apparent between children with ARND and pFAS ($p > 0.05$), nor for children with FAS and pFAS ($p > 0.05$).

3.3.5 Age

To examine the effect of age, children in the two experimental groups (controls and FASD) were distributed into different age bins: 8-10 years, 11-12 years, and 13-15 years. The ANOVA revealed a significant effect of age for SRT $F(2,165) = 11.2$, $p < 0.001$; CV $F(2,165) = 9.6$, $p < 0.001$; and direction errors $F(2,165) = 13.5$, $p < 0.001$; but not for express saccades ($F(2,165) = 0.4$, $p = 0.6$). Consistent with previous studies (Munoz et al., 1998), performance in these tasks improved across the range of ages tested for children with FASD and controls, as observed for antisaccade SRT and percentage of direction errors in the gap and overlap conditions (Fig. 3.4); and children with FASD differed significantly from control subjects across each age bin ($p < 0.05$). The same observations were made for antisaccade CV, as well as, prosaccade SRT, CV and percentage of direction errors (not shown). However, there was no interaction between age and group, suggesting that deficits in oculomotor control in children with FASD cannot be explained by developmental delay alone, as they failed to achieve age-matched control levels of performance.

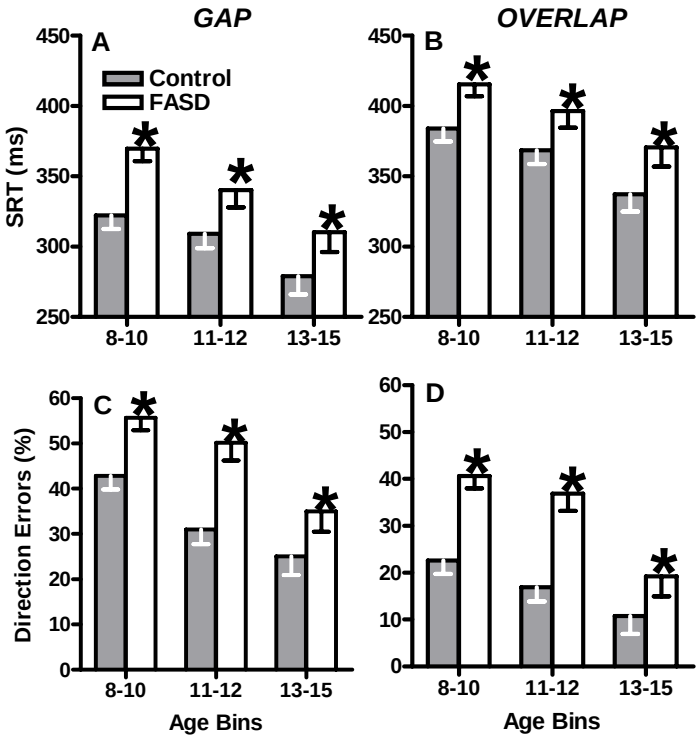

Figure 3.4. Mean saccadic reaction times (A,B) and direction errors (C,D) versus age for the antisaccade task in the gap (A,C) and overlap (B,D) conditions. Shaded bars, control data; open bars, fetal alcohol spectrum disorders (FASD) data. *$p \leq 0.05$ compared with control subjects.

3.4 Discussion

In this study, subjects were required to look either toward (prosaccade) or away from (antisaccade) a peripheral target that appeared on a computer monitor, which probes the ability of subjects to generate automatic visually triggered saccades, and to suppress the automatic saccade and generate a voluntary response in the opposite direction, respectively. Children with FASD exhibited increased saccadic reaction times, intra-subject variability, and direction errors. We also demonstrated that across the diagnostic subgroups, the greatest magnitude of difference in performance occurred for antisaccade tasks, reflecting deficits in executive function (Munoz and Everling, 2004). Moreover, across the ages examined, children with FASD never achieved a level of performance equivalent to the age-matched control group, suggesting that deficits in eye movement control may persist into adulthood (Chudley et al., 2007). We discuss these findings as they relate to the current understanding of oculomotor control and diagnostic subgroups of FASD.

3.4.1 Oculomotor Circuitry

The oculomotor system has been well characterized (Sweeney et al., 2007;Pierrot-Deseilligny et al., 2004;Munoz and Everling, 2004;Heide and Kompf, 1998). The main cortical areas involved in saccade generation are the parietal eye field (PEF) located in the posterior parietal cortex (PPC), as well as the frontal eye fields (FEF), the dorsolateral prefrontal cortex (dlPFC) and the supplementary eye fields (SEF) in the frontal lobe (Munoz and Everling, 2004), all of which project directly to the intermediate layers of the superior colliculus (SCi) to control saccade production. Oculomotor areas of the frontal cortex also send projections to the SCi via the *direct*, *indirect* and *hyperdirect* pathways

through the basal ganglia (Munoz et al., 2007;Hikosaka et al., 2000;Munoz and Everling, 2004;Nambu et al., 2002). The basal ganglia are generally associated with cognitive and motor function, and play a key role in oculomotor control (Hikosaka et al., 2000). The caudate nucleus is related to oculomotor behaviors that are necessary for predicting environmental changes (Hikosaka et al., 1989;Cameron et al., 2007), and decreased activity may impede performance even in simple oculomotor tasks such as the pro- and antisaccade tasks.

PEF lesions produce increased prosaccade latencies, with little effect on volitional saccades in monkeys (Lynch and McLaren, 1989); and unilateral lesions to the PPC increase prosaccade latency in both the gap and overlap conditions in humans (Pierrot-Deseilligny et al., 1987;Pierrot-Deseilligny et al., 1991). Patients with lesions to the FEF demonstrate profound difficulties in initiating antisaccades leading to elevated SRTs (Rivaud et al., 1994;Gaymard et al., 1999), suggesting its critical role in the initiation of intentional voluntary saccades. Lesions to the dlPFC lead to an increase in direction errors (i.e. automatic prosaccades) in the antisaccade paradigm; however, prosaccades are relatively unaffected (Pierrot-Deseilligny et al., 1991;Guitton et al., 1985). The SEF is important for saccade sequences by combining or coordinating voluntary saccades, and may be important for generation of successful antisaccades (Schlag-Rey et al., 1997;Amador et al., 2004).

The results from our study demonstrate two areas of deficient oculomotor control in children with FASD: 1) saccade initiation leading to increased SRTs; and 2) saccade suppression resulting in increased direction errors in the antisaccade task. These deficits are consistent with damage to parietal and frontal cortices, and basal ganglia. Structural

MRI studies have demonstrated a number of abnormalities following prenatal alcohol exposure: 1) a disproportionate reduction of activity in the parietal lobe (Archibald et al., 2001); 2) a relative increase in gray matter and decrease in white matter in the perisylvian cortex of the parietal lobes (Sowell et al., 2001); 3) reduced brain growth in ventral aspects of the frontal lobes (Riley et al., 2004); and 4) decreased basal ganglia volumes, with specific reductions in the caudate nucleus (Mattson et al., 1996;Archibald et al., 2001). Decreased caudate activity has also been shown using the blood oxygenated level dependent signal from fMRI in subjects with FASD following tasks that require inhibitory control (Fryer et al., 2007). Taken together, these findings indicate that prenatal alcohol exposure has prolonged effects on brain development long after the *in utero* insult. These results are consistent with the known deficits in executive function associated with FASD (Rasmussen, 2005), and implicate the parietal and frontal cortices, and basal ganglia as areas of particular sensitivity to prenatal ethanol exposure.

To summarize, PPC damage likely contributes to increased SRTs observed for prosaccades in children with FASD, while damage to frontal structures (FEF, SEF, dlPFC) and basal ganglia lead to increased SRTs for antisaccades and reduced ability to suppress automatic saccades. Downstream structures such as the SCi are likely affected only indirectly via aberrant projections from the frontal or parietal cortices, or basal ganglia. Based on the near-normal prosaccade metrics in FASD (Green et al., 2007c) and the normal gap-effect (this study), it appears that the SCi and brainstem saccade generating circuit remain structurally intact, and the functional abnormalities are due to atypical connections arising from upstream structures. We attribute the increased direction errors observed in the prosaccade task to difficulties in focused attention in

children with FASD. Future functional imaging studies using the same oculomotor tasks will confirm or refute the extent of involvement of these structures, and provide more definitive answers.

In contrast to our previous report (Green et al., 2007c), children with FASD did not execute fewer express saccades compared to controls. This observation was not attributed to sudden performance improvement by the children with FASD; rather it was due to the control subjects, who generated fewer express saccades under the experimental conditions used in the mobile laboratory. In our previous study complete darkness was achieved during experimental testing; however, during target presentation, the same conditions were not possible using the mobile laboratory and presence of ambient lumination likely underlie this result. These observations warrant further investigation.

3.4.2 Developmental Delay and FASD Subgroups

This large scale study allowed us to address questions related to the effects of age and diagnostic subgroup on oculomotor behavior in children with FASD. There was no age by group interaction in performance of the oculomotor tasks. Although there was an improvement with age, subjects with FASD failed to achieve age-matched control levels of task performance at any of the ages tested. This suggests that the deficits in oculomotor control cannot be explained by developmental delay alone, and are likely attributed to persistent brain injury well into adulthood (Chudley et al., 2007) and involving dysfunction of the frontal-striatal circuitry.

We postulated that eye movement testing would reveal differences in the magnitude of deficits among the diagnostic subgroups (i.e., FAS, pFAS, ARND). That is, we expected that children with FAS, considered to be at the more severe end of the

spectrum, exhibit the greatest magnitude of deficits in eye movement control. However, this was not the case, as children with ARND (i.e., not exhibiting facial dysmorphology of FAS) were not different from pFAS or FAS on the majority of outcome measures. Consistent with this observation, other published studies have reported no performance differences between dysmorphic and non-dysmorphic children, who were prenatally exposed to alcohol, on a number of neuropsychological tests that probe aspects of executive function (Mattson et al., 1999;Schonfeld et al., 2006). Alcohol-exposed individuals with and without facial features both exhibited statistically significant increases in cortical thickness demonstrating that the facial phenotype was not a prerequisite for brain dysmorphology (Sowell et al., 2008). Response inhibition in children and adults with heavy prenatal alcohol exposure showed no significant differences in the regions of interest between individuals with and without a FAS diagnosis, though both groups were significantly different from control subjects (Fryer et al., 2007). Of interest, in the clinical situation, it is the children with ARND that are most difficult to diagnose, as they lack the facial dysmorphology (Chudley et al., 2005). Although, we did not find differences between the diagnostic subgroups, all subgroups were different from their age-matched controls on multiple outcome measures, even the children with ARND (Figs. 3.2 and 3.3). Thus, measuring deficits in eye movement control may have significant potential for screening individuals at risk for FASD.

3.4.3 Conclusion

Saccadic eye movement tasks show promise for assessing the brain injury resulting from prenatal exposure to alcohol. Children between the ages of 8-15 years demonstrated profound deficits across many outcome measures for both pro- and

antisaccade tasks suggesting dysfunction in frontal and parietal cortices and the basal ganglia. Thus, eye movement experiments, and particularly the antisaccade task, provide objective measures of executive dysfunction in children with FASD, and may provide a more sensitive measure of overall cognitive function. This is an important point, as it has been shown that performance across tasks of executive function was lower in FASD than would be otherwise predicted by IQ alone, supporting the need for novel tools that can provide sensitive and specific assessments of brain injury (Niccols, 2007). With the advent of eye tracker systems equipped for use in MRI, it will be possible to identify the specific cortical and subcortical regions underlying these deficits.

Chapter 4 EXECUTIVE FUNCTION DEFICITS IN CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDERS (FASD) MEASURED USING THE CAMBRIDGE NEURO-PSYCHOLOGICAL TESTS AUTOMATED BATTERY (CANTAB)

4.1 Introduction

A serious and debilitating consequence to maternal consumption of alcohol during pregnancy is FAS, characterized by growth restriction, craniofacial dysmorphology and CNS dysfunction (Astley and Clarren, 2000;Clarren and Smith, 1978). However, FAS represents only a fraction (10-15%) of the children affected by prenatal exposure to alcohol; as it is more common for children to present with complex behavioural and neurological dysfunction related to their exposure, but in the absence of some or all of the characteristic facial features (Koren et al., 2003). In these situations, the diagnostic terms partial FAS (pFAS) and alcohol-related neurodevelopmental disorders (ARND) have been used to describe individuals who do not meet all of the criteria for FAS (Chudley et al., 2005;Stratton et al., 1996).

Recently, the term fetal alcohol spectrum disorders (FASD) has been widely adopted and encompasses all diagnoses and clinical presentations arising from prenatal alcohol exposure, including FAS (Koren et al., 2003;Chudley et al., 2005). Despite the various efforts to streamline diagnostic criteria, the need for accurate and objective measurement tools that could assist in the identification of individuals with prenatal exposure to alcohol is needed (Rasmussen, 2005). With early diagnosis, the appropriate clinical and therapeutic interventions could be implemented, with the goal of preventing and/or reducing the incidence of secondary disabilities (Streissguth et al., 2004;Streissguth et al., 1985;Streissguth et al., 1991).

A hallmark feature of FASD is deficits in executive function [see reviews (Rasmussen, 2005;Kodituwakku, 2007;Riley and McGee, 2005)]. Executive function is a heterogeneous term that refers to a range of abilities involved in conscious, goal-oriented behaviour (Funahashi, 2001). It has been postulated that a continuum exists wherein those with prenatal alcohol exposure, but without FAS, demonstrate less severe deficits compared to those with FAS. However, many research groups have failed to demonstrate differences in executive function between dysmorphic and non-dysmorphic individuals with prenatal alcohol exposure. For example, in a battery of 9 different executive function tests, Connor and colleagues (2000) found no relationship between executive function performance and facial phenotype. Task performance by dysmorphic and non-dysmorphic subjects was intermingled, suggesting that the presence of facial features was not predictive of performance. Similarly, both Schonfeld and colleagues (2006) and Mattson et al. (1999) failed to find significant differences in executive function among subjects with a history of prenatal alcohol exposure, both with and without facial features.

Recently, Kodituwakku postulated a behavioural phenotype for FASD associated with the existing patterns of deficits in cognitive-behavioural function (Kodituwakku, 2007). These deficits contribute to a wide range of negative life outcomes that include difficulties in academic, social and emotional aspects of life. Delineating the profile for FASD is difficult as the severity of alcohol effects varies widely as a function of exposure (i.e., quantity and frequency) and maternal factors (i.e., age, body weight) (Abel, 1995;Jacobson et al., 1998;Riley and McGee, 2005). However, individuals with FASD experience greater difficulty achieving complex adaptive behaviours that involve the

integration of multiple domains, and which depend on different parts of the brain; particularly the frontal lobes.

Interestingly, patients with FASD demonstrate clinical behaviours that resemble those for patients diagnosed with frontal lobe lesions (Connor et al., 2000); and a correlation between deficits in executive function and frontal lobe damage has been evaluated in the FASD population. Functional magnetic resonance imaging (fMRI) assessed inhibitory control in children and adolescents with prenatal alcohol exposure using the blood-oxygen-level-dependent signal (Fryer et al., 2007). FASD individuals demonstrated increased activation in the prefrontal cortex during trials that required inhibition of action compared to control subjects, suggesting that greater cognitive resources were required to perform the task. Increased neocortical thickness also has been found in individuals with FASD following structural MRI analysis, over large areas of the dorsolateral prefrontal lobes, especially in the right hemisphere (Sowell et al., 2008). Neocortical thickness has been associated with functional integrity, where neocortical thinning is associated with better general intellectual function. This is consistent with previous observations demonstrating increased neocortical thinning from childhood to adolescence (Sowell et al., 2004;Shaw et al., 2006). The correlation between a thin neocortex and better performance in control subjects was not found in the FASD group (Sowell et al., 2008), suggesting that pruning and myelination processes may not be occurring normally in children with prenatal alcohol exposure. This disorganization of neocortical structures may result from cellular changes that occur during postnatal development and that are impacted by heavy prenatal alcohol exposure;

thus, supporting the view that brain-behaviour relationships do not develop normally in these individuals.

The aim of our study was to assess executive function in children with FASD compared to age- and sex-matched controls, using the Cambridge Neuropsychological Test Automated Battery (CANTAB®) computerized test battery. Investigators have used CANTAB® tasks to assess executive function during typical development (Luciana and Nelson, 1998), and in other neurodegenerative (Egerhazi et al., 2007) and neurodevelopmental disorders (Goldberg et al., 2005). The CANTAB® has several advantages over other measures of executive function, as it provides a standardized computer-administration format that controls for variations among different examiners. It is nonverbal and provides information about direction and accuracy on screen and employs the use of a touch-screen response that automates data acquisition. Finally, there is evidence for the involvement of prefrontal and medial temporal brain regions in the performance of the CANTAB® tasks (Luciana and Nelson, 2002). Thus, it was hypothesized that children with FASD exhibit deficits in different domains of executive function that can be quantified with the CANTAB® battery of neuropsychological tasks. Second, it was hypothesized that tasks which demand the use of spatial working memory and strategy demonstrate the most sensitivity to deficits in cognitive flexibility in children with FASD, as these functions are particularly deficient in these individuals. Finally, based on the literature, we also hypothesized that the magnitude of deficits in task performance in CANTAB® is not different among the FASD diagnostic subgroups, with and without the facial dysmorphology (i.e., FAS vs pFAS vs ARND).

Preliminary versions of these data has been presented in abstract form (Mihic et al., 2007;Green et al., 2007a).

4.2 Materials and Methods

4.2.1 Participants

All experimental procedures were reviewed and approved by the Human Research Ethics Boards of Queen's University, the Children's Hospital of Eastern Ontario (Ottawa subjects), and the University of Alberta (Edmonton subjects). Parents provided informed consent for participation. Children with FASD were recruited from 8 different communities across Ontario and 1 from Alberta, and were previously assessed at local diagnostic clinics and in accordance with the Canadian Diagnostic guidelines (Chudley et al., 2005). A total of 189 subjects were recruited into the study: 92 (40 males, 52 females; 11.2 ± 0.2 years of age, range 8-15 years) were control children (non-FASD) and 97 were grouped as FASD. Of the 97 subjects included in the FASD group, 89 (44 males, 45 females; 10.7 ± 0.2 years of age, range 8-15 years) had a diagnosis within the FASD spectrum (FAS, pFAS, ARND), while 8 were suspected and/or exposed, but had yet to receive a definitive diagnosis. All data included in the analysis for the FASD group were obtained from the 89 children who had received a diagnosis within the spectrum.

Of the 89 children with FASD, 60 were medicated (Table 4.1) for behavioural symptoms relating to their co-morbidities (Table 4.2). On the test day, primary caregivers were asked to withhold stimulant medication until the testing was completed. Of the 60 children taking medications, 8 were tested on medication (87% compliance with the off medication request), in most cases because the primary caregiver judged that the child would not be able to complete testing off medication. For the remaining 52 children, their last daily dose of stimulant medication was administered a minimum of 12

Table 4.1 Medication history for children with FASD and Controls.

Medications	**FASD**	**Control**
Stimulants	38 (42.7%)	0 (0%)
Antipsychotics	29 (32.6%)	0 (0%)
Antidepressants	10 (11.2%)	0 (0%)
Anticonvulsants	3 (3.4%)	0 (0%)
Other*	20 (22.5%)	12 (13%)

*Antihistamine, Anti-asthma, Oral contraceptives, Melanin

Table 4.2 Comorbidity history for children with FASD and Controls.

Comorbidities	**FASD**	**Control**
sleeping disorders	55 (61.7%)	10 (10.9%)
Attention deficit hyperactivity disorder (ADHD)/attention deficit disorder (ADD)	53 (59.6%)	0 (0%)
oppositional defiant disorder	19 (21.3%)	0 (0%)
anxiety	15 (16.9%)	0 (0%)
asthma	10 (11.2%)	12 (13.0%)
depression	10 (11.2%)	1 (1.1%)
neurological disorder	8 (8.9%)	0 (0%)
bipolar	6 (6.7%)	0 (0%)
seizure	5 (5.6%)	3* (3.3%)
mood disorder	5 (5.6%)	0 (0%)
conduct disorder	3 (3.4%)	0 (0%)
astigmatism	2 (2.3%)	3 (3.3%)
allergies	2 (2.3%)	3 (3.3%)
autism	2 (2.3%)	0 (0%)
Asperger's disorder	2 (2.3%)	0 (0%)
psychosis	2 (2.3%)	0 (0%)
pre-depression	1 (1.1%)	0 (0%)
hearing aids	1 (1.1%)	0 (0%)
myopia	1 (1.1%)	3 (3.3%)
reactive attachment disorder	1 (1.1%)	0 (0%)
anger management	1 (1.1%)	0 (0%)
chronic ear infection	1 (1.1%)	1 (1.1%)
twisted femur	1 (1.1%)	0 (0%)
kidney problems	1 (1.1%)	0 (0%)
heart problems	1 (1.1%)	0 (0%)
hepatitis C	1 (1.1%)	0 (0%)
lactose intolerance	1 (1.1%)	0 (0%)
Tourette's	1 (1.1%)	0 (0%)
hypotonia	1 (1.1%)	0 (0%)
Migraines	0 (0%)	1 (1.1%)
Scoliosis	0 (0%)	1 (1.1%)
Strabismus	0 (0%)	1 (1.1%)

*One time events, no diagnosis of seizure disorder/epilepsy

hours prior to testing.

All control subjects had no known neurological, psychiatric or visual disorders, other than requiring corrective lenses. Primary caregivers were informed of the nature of the study and provided written consent on behalf of the participants. All subjects completed one 30-45 minute neuropsychological battery. Each subject received $10 and a small gift for participating in the study.

4.2.2 Neuropsychological Battery: CANTAB®

Subjects were asked to complete a series of 4 computerized neuropsychological tests of the CANTAB® (Cambridge Cognition, Cambridge, United Kingdom). Subjects were seated in front of a laptop screen and were instructed to carry out the tests by either touching the screen or by pressing/releasing a press pad. Children were given short breaks whenever needed, and snacks and beverages were provided upon completion of the neuropsychological battery.

The goal of this research study was to assess the following four domains of executive function: attention, planning, strategy use and spatial working memory. Based on these areas of interest, the following four tests were selected as they were both age- (other tasks were considered too difficult for the younger children in our cohort) and time-appropriate (each testing session could be completed in 45 minutes or less): Reaction Time (RTI) (which was used as a measure of attention and as a simple motor screening task), Stockings of Cambridge (SOC) (assessed planning and strategy), Match to Sample Visual Search (MTS) (assessed attention) and Spatial Working Memory (SWM) (assessed spatial working memory and strategy). These tests will be described briefly, as well as the pertinent outcome measures. Some children with FASD had

difficulty completing the full battery set (9 did not complete the SOC task; 2 did not complete the MTS or SWM tasks), and in one situation a computer error prevented the recording of data from one test for one control subject (SWM task).

4.2.2.1 Reaction Time (RTI)

In the RTI task, subjects were instructed to press down on the press pad until a yellow dot appeared in the centre of either a single circle (simple) or in one of 5 different concentric circle locations (5-choice). Upon appearance of the yellow dot, the subject was instructed to release the press pad and touch the yellow circle as quickly as possible. The outcome measures were reaction time (time to release press pad after the yellow circle appeared) and movement time (time to touch the screen after releasing the press pad) for both the simple and 5-choice problem sets.

4.2.2.2 Stockings of Cambridge (SOC)

The SOC task is similar to the Tower of London task, a derivative of the classic Tower of Hanoi (Shallice, 1982), with the advantage being that data collection is automated. Each problem set was comprised of three "stockings" or socks suspended from a beam of different lengths containing different coloured balls (green, red and blue). The computer created a problem set in the top portion of the screen and subjects were then asked to copy this same configuration by moving their coloured balls so that the two problem sets were identical. As the minimum number of moves increased, the complexity of the problems increased as well. Outcome measures included the problems solved in the minimum number of moves, the mean number of moves for each n-move problem (i.e., n=2, 3, 4 or 5) and the mean initial thinking time for each n-move problem. The SOC task also employed two modes for evaluation; one was a *copy* mode and one

was a *follow* mode. In the *copy* mode subjects were required to copy the problem set in the required number of moves, while the *follow* mode instructed subjects to follow the balls as they were moved into different stockings. The initial mean thinking time measures were determined by taking the difference between the *copy* and *follow* mode, which controlled for motor deficits.

4.2.2.3 Match to Sample Visual Search (MTS)

In the MTS task, a red box appeared in the centre of the screen concentrically surrounded by 8 empty boxes. Upon pressing down on the press pad, a pattern appeared in the center box, and after a brief delay an array of patterns appeared in 2, 4 or 8 of the surrounding boxes. Upon recognition of the matching pattern, the subject was instructed to release the press pad and touch the pattern that matched the center pattern. The outcome measures included decision and movement times for each n-choice (i.e., n=2, 4 or 8) problem. Decision time represented the time to release the press pad after the distracter patterns appeared, and the movement time was the time from releasing the press pad to touching the correct pattern on the screen.

4.2.2.4 Spatial Working Memory (SWM)

In the SWM task subjects were presented with randomly distributed coloured boxes ranging in number from 4 to 8. Subjects were instructed to locate hidden tokens that appeared under each coloured box and move them to fill an empty panel located on the right-hand side of the screen. Once a token had been located, subjects had to recall that the computer would never hide a token in a coloured box previously found to contain one; therefore, they had to remember *not* to revisit those coloured boxes. The outcome measures analyzed were total number of errors (returning to a box previously found to

contain a token), errors for each n-box problem (i.e., n=4, 6 or 8), and a strategy score, which indicated the use of a search strategy by the subject (low scores indicated good strategy use).

4.2.3 Data Analysis

All dependent measures (RTI: reaction and movement time; SOC: problems solved in the minimum number of moves and initial thinking time; MTS: movement time and decision time and SWM: errors and strategy score) were analyzed using ANOVA with α set at 0.05. Two-tailed, unpaired Student's *t*-tests were conducted and corrected with Welch's approximation when the assumption for homogeneity of variance was not met (SOC: initial thinking time across problem sets; MTS: movement and decision time across problem sets). Data was analyzed by non-parametric Mann-Whitney tests when the conditions for normal distribution were not met. The effect of diagnosis (ARND, pFAS and FAS) also was determined by matching each subject in the FASD group (as closely as possible), to a control by age and sex. FASD and control subjects once subdivided were analyzed by univariate analysis to demonstrate differences. Effect sizes were calculated from the means and standard deviations obtained for the major outcome measures (Cohen, 1988). We focus on descriptions of the relevant statistical parameters for comparisons that occurred between the control and FASD groups.

4.3 Results

We separately analyzed the impact of several potential confounding variables in this study. First, 8 children in the FASD group were tested on stimulant medication. However, when the data for these children were excluded from the analysis there was no substantive change in the overall performance of the FASD group reported in this communication. Second, within the FASD group, we compared the performance of children withdrawn from stimulant medication to those children who were not taking any stimulant medication. There were no differences between these groups for any of the outcome measures reported in this communication (data not shown), suggesting that stimulant drug withdrawal had a negligible impact on performance of the CANTAB® tasks. Third, the major co-morbidity for children with FASD was attention-deficit hyperactivity disorder (ADHD) (Table 4.2), and therefore we conducted an analysis of performance between children who were co-morbid for ADHD versus those that were not. There were no differences between these groups for any of the outcome measures reported in this communication (data not shown), suggesting that the co-morbid diagnosis of ADHD was not the major contributor to performance deficits in the CANTAB® tasks.

4.3.1 Reaction Time (RTI)

Figure 4.1 depicts the four outcome measures for the RTI: Simple and 5-Choice reaction and movement times. The univariate ANOVA revealed the following for dependent measures (simple and 5-choice reaction and movement times) and between-subject measure (group). As compared to controls, children with FASD demonstrated increased reaction times in the simple choice ($F(1,169) = 14.5$, $p < 0.001$) and 5-choice

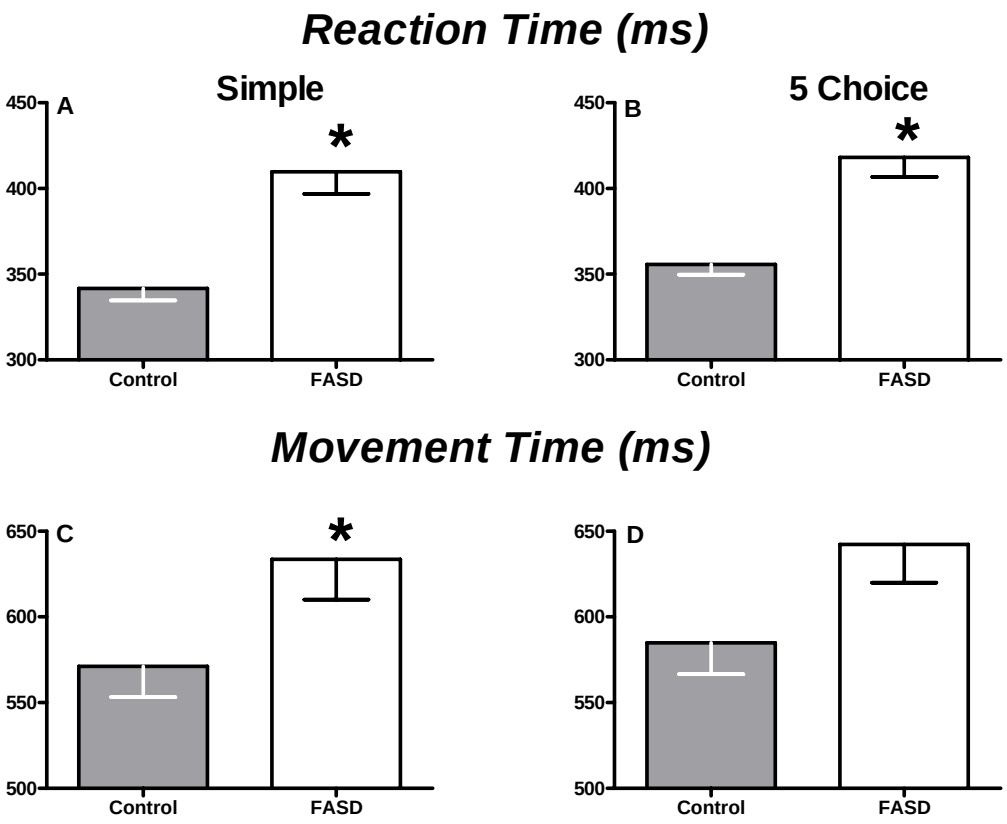

Figure 4.1 Quantification of parameters for reaction time (A,B) and movement time (C,D) for simple (A,C) and 5-choice (B,D) problems in the Reaction Time (RTI) task. *$p < 0.05$ compared with control subjects.

($F(1,169) = 16.2$, $p < 0.001$) tasks (Fig. 4.1A, B); as well as, increased movement times in the simple choice ($F(1,169) = 4.6$, $p < 0.05$) task (Fig. 4.1C) and a similar trend for the 5-choice ($F(1,169) = 3.0$, $p = 0.08$) tasks (Fig. 4.1D).

4.3.2 Stockings of Cambridge (SOC)

Of the outcomes measures available for SOC, the problems solved in the minimum number of moves, mean moves and initial thinking time were selected for analysis (Fig. 4.2). The univariate ANOVA for the dependent measure (problems solved in the minimum number of moves) and for between-subject effect (group) revealed that children with FASD solved fewer problems in the minimum number of moves ($F(1,160) = 10.2$, $p < 0.005$) (Fig. 4.2A) than the control group. Figure 4.2B illustrates the breakdown in performance for children with FASD compared to controls for problem sets of increasing difficulty. These data were analyzed using the non-parametric Mann-Whitney test because the conditions for normal distribution were not met. Children with FASD demonstrated poorer performance (i.e., more moves to solve a given problem) for 2-move ($p < 0.05$), 4-move ($p < 0.05$) and 5-move ($p < 0.05$) problems compared to control children. For initial thinking time, the ANOVA for dependent measures (choice; 2, 3, 4 or 5), revealed an effect of choice ($F(3,399) = 12.8$, $p < 0.001$) such that the children with FASD spent less time planning a strategy for completing the problem sets. Children with FASD demonstrated a significant decrease in their initial thinking time for 4-move ($t_{(165)} = 1.9$, $p = 0.05$) and 5-move ($t_{(156)} = 2.1$, $p < 0.05$) problem sets compared to control children, and no significant difference for 2- and 3-move problems (Fig. 4.2C).

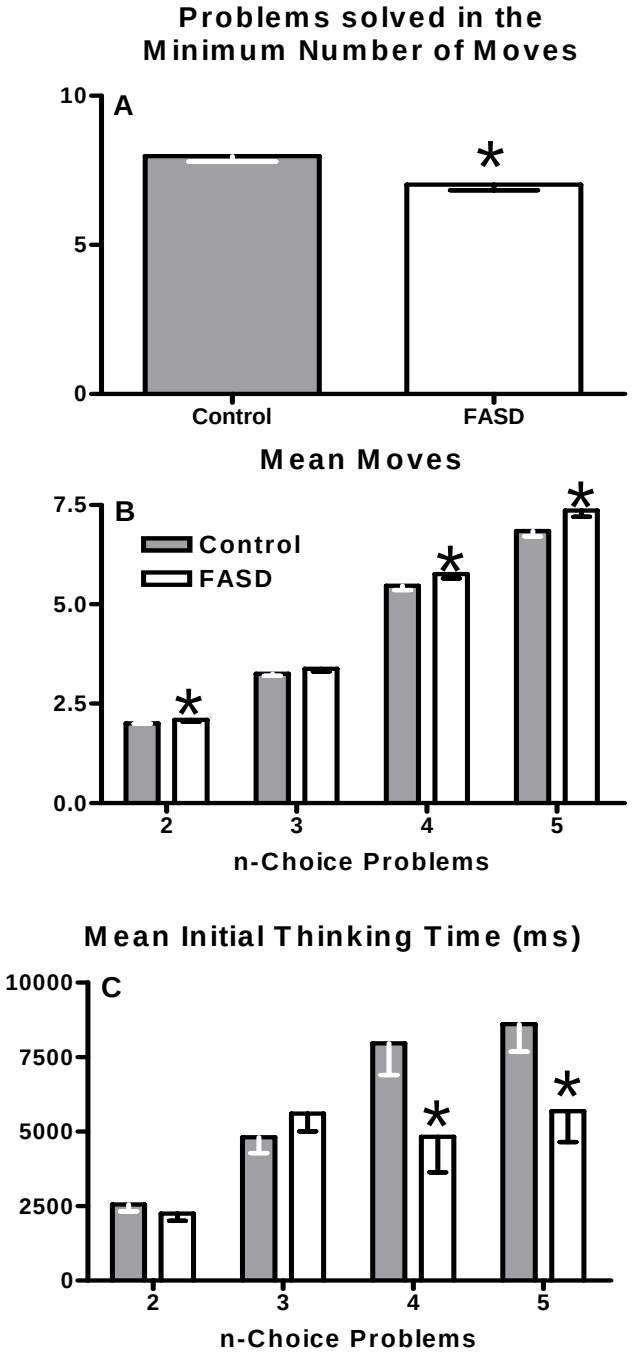

Figure 4.2 Quantification of parameters for the total problems solved in the minimum number of moves (A), number of moves for n-choice problems (B) and mean initial thinking time for n-choice problems (C) in the Stockings of Cambridge (SOC) task. Open bars, fetal alcohol spectrum disorders (FASD) data; Shaded bars, control data. *$p < 0.05$ compared with control subjects.

4.3.3 Match to Sample Visual Search (MTS)

Figure 4.3 displays the outcome measures for MTS. For the univariate ANOVA of the dependent measure mean movement time for correct responses, the effect of group approached significance ($F(1,167) = 3.0$, p = 0.08); children with FASD were slower than controls (Fig. 4.3A). For the decision time for correct responses, there was a group effect ($F(1,167) = 12.4$, $p < 0.005$), demonstrating that children with FASD were slower to recognize and decide which distracter pattern matched the central stimulus pattern compared to controls (Fig. 4.3C). The movement and decision times across problem sets of increasing difficulty are depicted in Fig. 4.3B and D. Movement time increased with increasing difficulty (i.e., increased box number) and the ANOVA revealed for the dependent measure (boxes; 2, 4, 8) an effect of box ($F(2,332) = 29.1$, $p < 0.001$). The group effect approached statistical significance ($F(1,166) = 2.8$, p = 0.1); where children with FASD had slower movement times compared to control children (Fig. 4.3A). Decision time increased as the problem sets became more difficult (i.e., increased box number) and the ANOVA revealed a significant effect of box ($F(2,332) = 198.3$, $p < 0.001$). There was a group effect for decision time ($F(1,166) = 13.6$, $p < 0.001$); which demonstrated that children with FASD took longer to decide which distracter pattern matched the central target pattern (Fig. 4.3C). For movement time, children with FASD demonstrated significant motor delays for both the 4- and 8-box problem sets ($t_{(99)} = 2.5$, $p < 0.05$ and $t_{(116)} = 2.1$, $p < 0.05$, respectively); and this approached significance in the 2-box problem set ($t_{(103)} = 1.8$, p = 0.08) (Fig. 4.3B). Similarly, for decision time, children with FASD required more time to differentiate the matching pattern from distracters for

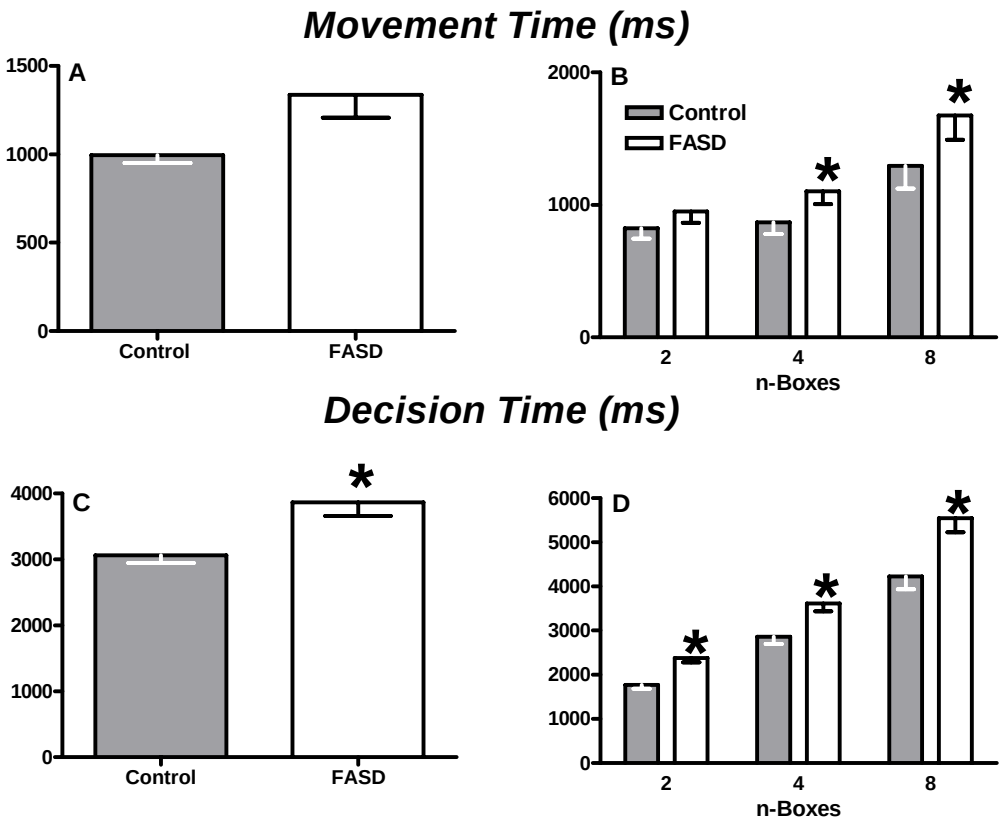

Figure 4.3 Quantification of parameters for the total movement time (A) and decision time (C); and the total movement time (B) and decision time (D) for n-move problems in the Match to Sample Visual Search (MTS) task. Open bars, fetal alcohol spectrum disorders (FASD) data; Shaded bars, control data. *$p < 0.05$ compared with control subjects.

2-, 4- and 8-box problems sets ($t_{(136)} = 4.4$, $p < 0.001$, $t_{(150)} = 3.0$, $p < 0.005$ and $t_{(134)} = 3.1$, $p < 0.005$, respectively) (Fig. 4.3D).

4.3.4 Spatial Working Memory (SWM)

Figure 4.4A depicts the total number of errors (when a subject revisited a box previously found to contain a token) in the SWM task for control and FASD subjects. The univariate ANOVA for the total number of between errors revealed a significant difference for group where children with FASD committed more errors compared with controls ($F(1,166) = 44.4$, $p < 0.001$). Figure 4.4B depicts the number of errors stratified by increasing task difficulty (i.e., increasing the number of boxes). As in the SOC task, the conditions for normal distribution were not met and as a consequence non-parametric analysis was conducted using the Mann-Whitney test. There was a significant difference for the 4-, 6- and 8-box problem sets ($p < 0.001$), indicating that the children with FASD demonstrated deficits in working memory such that they were unable to recall which boxes had been previously searched and found to contain a token. This observation was further supported by examining the strategy score; where lower scores indicate good use of strategy (Fig. 4.4C). The univariate ANOVA revealed a significant effect of group on strategy score, such that the control children demonstrated significantly lower scores ($F(1,166) = 23.6$, $p < 0.001$).

4.3.5 Effect Size

The effect sizes were calculated for the major outcome measures obtained in the CANTAB® tasks (Table 4.3). In the RTI task, there were moderate to strong effects in reaction times, but relatively small effects in the movement time measures. Similarly, in the MTS task, there was a moderate effect on decision time, but a relatively small effect

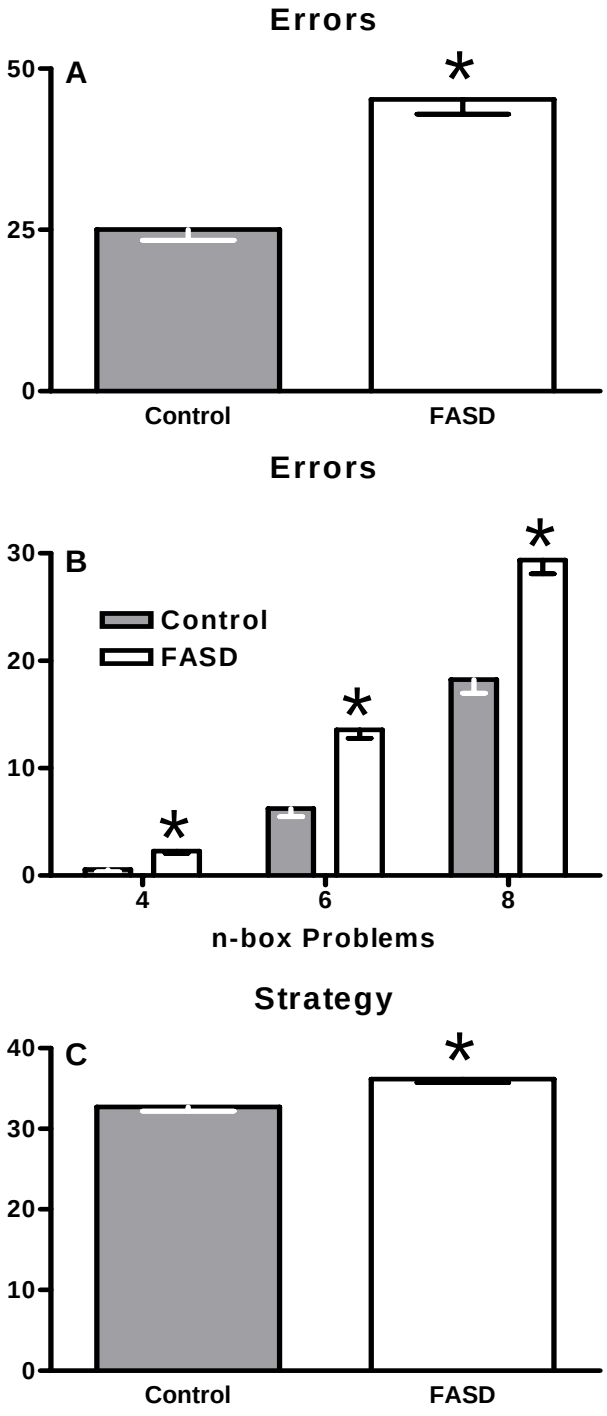

Figure 4.4 Quantification of parameters for the total errors (A) and errors for n-box problems (B); and the strategy score (C) in the Spatial Working Memory (SWM) task. Open bars, fetal alcohol spectrum disorders (FASD) data; Shaded bars, control data. *$p < 0.05$ compared with control subjects.

Table 4.3 Effect size for CANTAB outcome measures

Task	**Cohen's *d***	**Effect size *r***
RTI (Reaction Time-Simple)	0.70	0.33
RTI (Reaction Time-5 Choice)	0.73	0.34
RTI (Movement Time-Simple)	0.31	0.16
RTI (Movement Time-5 Choice)	0.30	0.15
MTS (Decision Time)	0.51	0.25
MTS (Movement Time)	0.37	0.18
SOC (Minimum Moves)	0.55	0.26
SWM (Errors)	1.08	0.48
SWM (Strategy)	0.75	0.35

on movement time. In the SOC task, the number of problems solved in the minimum number of moves yielded a moderate effect. In contrast, very strong effects were obtained in the SWM task, especially for the number of errors (searching a box previously found to contain a token).

4.3.6 Diagnostic Subgroups

In the FASD group, 26 children had a diagnosis of FAS, 18 children had a diagnosis of pFAS and 42 children had diagnosis of ARND. The control children were matched to children from 1 of 3 subgroups (paired to FAS, paired to pFAS or paired to ARND) based upon age and sex. Outcome measures for each task were then analyzed and stratified by diagnosis for FASD and control children (control-FAS, control-pFAS and control-ARND to signify the match). There was no significant difference between diagnostic subgroups for any of the outcome measures in the tasks RTI, MTS and SWM, although all diagnostic subgroups were different from controls (data not shown). In the SOC task, there was one significant difference found between children in the diagnostic subgroups ($F(2,70) = 5.2$, $p < 0.01$) that was not apparent for the matched control groups. Specifically, children with a diagnosis of FAS solved significantly fewer problems compared to children with a diagnosis of either pFAS ($p < 0.05$) or ARND ($p < 0.05$) (Fig. 4.5).

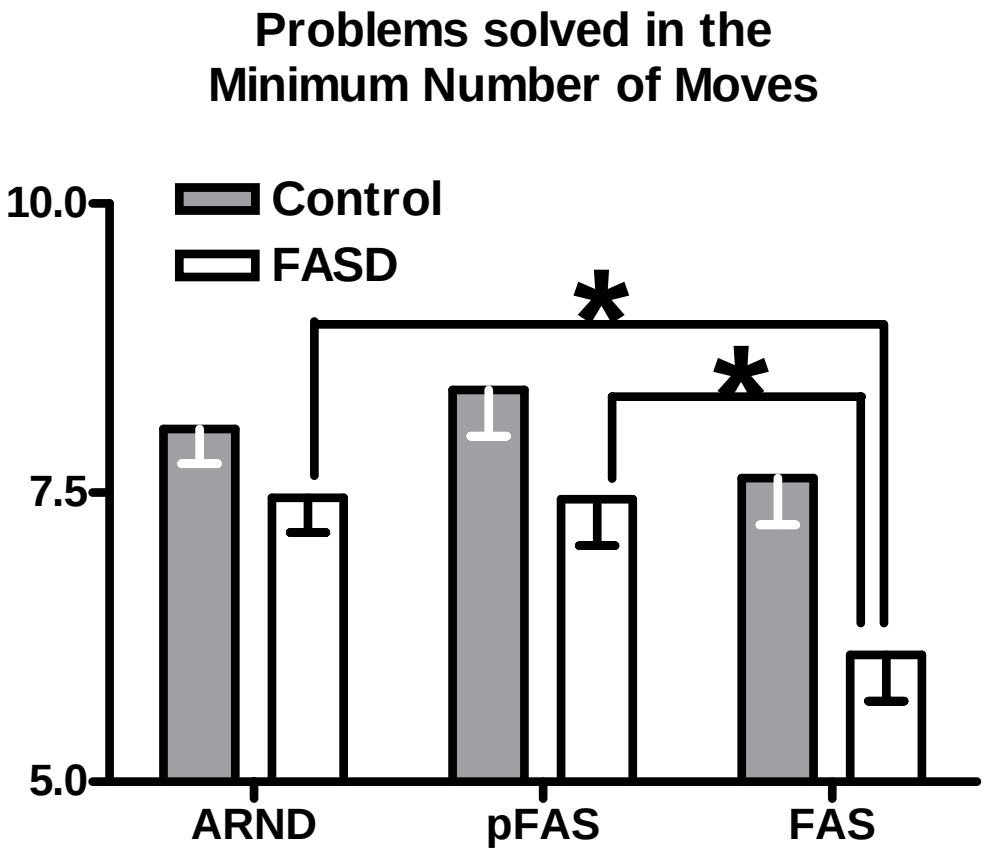

Figure 4.5 Quantification of the total problems solved in the minimum number of moves in the Stockings of Cambridge (SOC) task across diagnostic groups. Control subjects were matched perfectly or near perfectly to children with FAS, pFAS or ARND based on age and gender. Open bars, fetal alcohol spectrum disorders (FASD) data; Shaded bars, control data. *$p < 0.05$ depicts statistical significance.

4.4 Discussion

Our results suggest that the computerized CANTAB® research tool provides a sensitive indicator of executive function deficits in children with FASD. Compared to controls, children with FASD exhibited: 1) longer reaction/decision time latencies in the RTI and MTS tasks; 2) a decrease in the number of problems solved in the minimum number of moves, an increase in the mean number of moves and a decrease in the initial thinking time in the SOC task; 3) increased errors and poorer use of strategy in the spatial working memory task; and 4) little or no difference in performance across the diagnostic subgroups (FAS, pFAS, ARND). Performance in these tasks revealed deficits in attention, planning, strategy use and working memory. In this study population, the effect size for impaired performance by children with FASD was greatest for the spatial working memory task. These results support our initial hypotheses that children with FASD demonstrate deficits in executive function that can be measured experimentally, and that tasks that demand the use of spatial working memory and strategy demonstrate the most sensitivity to deficits in cognitive flexibility in children with FASD. Of the 14 outcome measures analyzed across the four selected tasks, only one revealed significant differences in performance across the diagnostic subgroups (FAS, pFAS, ARND), indicating that deficits in the different domains of executive function provide sensitive indicators of brain injury resulting from prenatal exposure to alcohol, regardless of the presence of facial dysmorphology.

Our findings are consistent with previous studies in which prenatal alcohol exposure has been correlated with generalized neurocognitive performance across tasks of executive function [see reviews (Niccols, 2007;Kodituwakku, 2007;Rasmussen,

2005;Riley and McGee, 2005)]. Korkman and colleagues (2003) found deficits in executive function and attention, and also demonstrated deficits in other cognitive domains (Korkman et al., 2003). Similarly, Lee et al. (2004) were able to classify alcohol-exposed children and controls with 93.3% and 90% specificity, respectively, using two common attentional outcome measures from the Wechsler Intelligence Scale for Children – Third Edition and the Attention Problems scale from the Child Behaviour Checklist (Lee et al., 2004). The selected measures for attention reliably predicted whether a child was prenatally exposed to alcohol, suggesting that attention deficits may warrant further investigation as a potential mechanism for differentiating children with FASD from other clinical populations.

The use of implicit strategy has been evaluated in children with heavy prenatal alcohol exposure to determine its effects on learning and memory (Roebuck-Spencer and Mattson, 2004). When semantic categories were not used, children with FASD forgot more information than controls on a verbal learning test, and the greater the use of semantic clustering positively correlated with the amount of information learned and recalled. Interestingly, these observations were not driven by IQ differences between the groups, suggesting that alcohol may affect memory abilities independent of its effect on intellectual ability. The use of semantic clustering and rehearsal may be suitable strategies for optimal learning in children with FASD. Recently, rehearsal training has been evaluated in children with FASD (Loomes et al., 2007). Similar to the current study, there were difficulties in the use of strategy and working memory in the children with FASD, and these performance deficits were exacerbated with increasing task complexity. However, children who received rehearsal training performed significantly

better than the control group and demonstrated the use of rehearsal strategies from their training sessions, thus improving overall memory capabilities. We did not employ rehearsal training in the current study, and thus it is possible that the poor performance for children with FASD can be attributed to their inability to effectively develop suitable strategies themselves in order to perform the tasks successfully. It is likely that the implementation of rehearsal training may help to improve these core deficits.

After reviewing the literature, it was not surprising that there was only one difference in cognitive performance among the diagnostic subgroups (FAS, pFAS and ARND). Previous studies have reported little to no differences among dysmorphic and non-dysmorphic subjects with prenatal alcohol exposure (Mattson et al., 1999;Roebuck-Spencer and Mattson, 2004;Kodituwakku et al., 2001); and this is consistent with results we obtained evaluating oculomotor control across the diagnostic subgroups (Green et al. 2008, unpublished findings). Individuals with FAS and fetal alcohol effects (FAE; describes children who do not have all the physical characteristics of FAS) do not show differences on tests of cognitive abilities, secondary disabilities and behavioural problems (Sampson et al., 2000). Furthermore, it has been suggested that central nervous system deficits in FAE may be as severe as or worse than individuals with FAS (Connor and Streissguth, 1996). Thus, it is not surprising that significant differences in performance among the three diagnostic subgroups were found only in one outcome measure.

While this study provided valuable data representative of a large sample size of children with and without FASD, there were some limitations that should be noted. First, we did not employ other neurocognitive tests in this study, which precludes the determination of the relative sensitivity of CANTAB® compared with more traditional

cognitive and behavioural assessment tools. Second, data on ethnicity was not collected in this study. As this may be associated with performance, differences in CANTAB® task execution by ethnic group should be addressed in future studies. Third, it was not possible to collect data regarding the frequency, quantity and timing of prenatal alcohol exposure for each of our subjects, as this information was unavailable, especially for children living in adoptive families or in foster care. It has proven very difficult to ascertain a dose-response relationship for alcohol exposure and outcome in offspring, as there are many factors (i.e., nutrition, genetics, ethnicity) that contribute to the severity, and for which we were unable to control. Fourth, information on alcohol and nicotine use was not quantified for our study population. It may be of interest to determine the frequency and types of drug use among children between the ages of 8-15 years, as this may impact on performance. Finally, as this was the first study to assess executive function in children with FASD using the CANTAB®, only a specific subset of tasks was selected. In future, tasks that probe other aspects of executive function may warrant further investigation, especially those which assess verbal learning.

In conclusion, brain injury resulting from prenatal alcohol exposure can lead to significant deficits in cognitive abilities that can be quantified using an easy to administer, brief battery of computer-based neuropsychological tasks. We demonstrate herein deficits in attention, planning, strategy and working memory, revealing that the neurocognitive problems associated with FASD are widespread and generalized; though deficits in spatial working memory may be affected to the greatest extent. Prenatal alcohol exposure has been related to a decrease in the size of frontal cortex (Wass et al., 2001;Riley et al., 2004;Sowell et al., 2008), which likely gives rise to most of these

impairments regardless of the presence or absence of facial dysmorphology. Finally, by understanding the global dysfunction and comparing the subtle differences among subjects with FASD to other clinical populations, it may be possible to develop specific strategies and techniques that may mitigate the brain injury resulting from prenatal exposure to alcohol and overcome these deficits in executive function.

Chapter 5 DEFICITS IN OCULOMOTOR CONTROL ARE CORRELATED WITH PERFORMANCE IN EXECUTIVE FUNCTION TASKS IN CHILDREN WITH FETAL ALCOHOL SPECTRUM DISORDERS (FASD)

5.1 Introduction

We have evaluated the use of saccadic eye movements as a novel method for assessing brain function in children with FASD. *Saccades* are fast movements, in which the line of sight is rapidly redirected as the eyes move in a coordinated fashion toward a target that appears in the peripheral hemifield (Leigh and Zee, 1999). They are generated quickly, and can be automatic or voluntary in nature depending on the task at hand. They are easy to measure and non-invasive, and have been used to characterize brain function in both neurodevelopmental and neurodegenerative disorders (Leigh and Kennard, 2004;Munoz et al., 2007;Ramat et al., 2007). Of the different types of saccades, it is the volitional saccades that assess voluntary control (Leigh and Zee, 1999;Munoz and Everling, 2004). They are internally triggered and provide a measure of both inhibitory control and voluntary eye movement. In the antisaccade task – a type of volitional saccade, the stimulus location and saccade goal are incompatible, and the automatic response (i.e., toward the peripheral target) must be inhibited and a voluntary saccade must be generated in the opposite direction (away from the peripheral stimulus) (Fig. 1.1). This task has been used to further characterize executive function in different clinical populations (Munoz and Everling, 2004;Ramat et al., 2007;Munoz et al., 2007).

Although the concept of executive function is used commonly in the literature, its definition remains more elusive. Executive function is generally accepted to represent abilities that involve higher-level cognitive functions that describe 'how' human behaviour is expressed (Lezak, 1995). Early lesion studies of individuals with frontal and

prefrontal lobe damage are credited with demonstrating their central role in executive function. These first observations suggested that only the frontal lobes were involved; however, it is now widely accepted that different subcortical and thalamic pathways also play an important role [see review (Jurado and Rosselli, 2007)]. To date, several neural circuits involving the frontal lobes, basal ganglia and thalamus have been implicated in executive function performance (Royall et al., 2002). The dorsolateral prefrontal cortex circuit underlies functions in planning, goal selection, set-shifting, working memory and self-monitoring; the lateral orbitofrontal circuit is involved in risk assessment and inhibition; and the anterior cingulate circuit monitors behaviour and self-correcting.

Deficits in executive function are a hallmark feature of FASD (Rasmussen, 2005), and have been studied extensively in different cohorts of FASD subjects (Loomes et al., 2007;Lee et al., 2004;Mattson et al., 1999;Roebuck-Spencer and Mattson, 2004;Schonfeld et al., 2001). These studies have provided valuable information correlating the adverse effects of alcohol on brain development and function; and many of these tools are used to assist in the diagnosis of individuals within the FASD spectrum. Recently, our laboratory has adopted a standardized, computerized neuropsychological test battery for testing children with FASD. The Cambridge Neuropsychological Test Automated Battery (CANTAB®) is a highly validated research tool that has been used to assess executive function in children (Luciana and Nelson, 2002). It has several advantages over previous measures of executive function, as it provides a standard computer-administration format that controls for variations among different examiners, and there is support for the involvement of prefrontal and medial temporal brain regions in the performance of the CANTAB® tasks (Luciana and Nelson, 1998).

Our laboratory has adapted both the eye movement experiments and neuropsychological battery for use as a mobile laboratory, with the advantage of attracting a greater number of participants into the study. The data generated in this study allowed us to test the hypothesis that deficits in eye movement control are correlated with performance in standardized neuropsychological tasks in children with FASD.

Preliminary versions of these data has been presented in abstract form (Green et al., 2007a).

5.2 Materials and Methods

5.2.1 Participants

The composition of the study group is described in detail in the preceding chapters. Additional information on the demographics of the study population is presented in Appendix F (Table F.1). Primary caregivers were informed of the nature of the study and provided written consent on behalf of the participants. All subjects completed one, 1-hour eye movement session and a 45-minute neuropsychological test battery. Each subject received $10 and a small gift for participating in the study.

5.2.2 Volitional Antisaccade Task

Participants performed two blocks of anti-saccades; each containing 80 trials (Fig. 1.1). For the antisaccade task, a red dot was positioned at the centre of the screen and served as the initial fixation point. Red target dots were positioned on the screen at 15^0 to the right or left of the central fixation point. The screen was diffusely illuminated between trials to avoid dark adaptation. Each trial began with a 250-ms period of darkness. In the task, participants were instructed to look away from the eccentric target to the opposite side. The target remained illuminated for 1,000 ms, after which all visual presentation disappeared and the background illumination reappeared, indicating the end of that trial. Target location (right or left) was pseudo-randomly interleaved throughout each block of trials. Subjects were asked to repeat and demonstrate the instructions to the experimenter to ensure that they understood the paradigm before the onset of data collection. For correlational analysis, the percentage of direction errors (saccades generated towards the peripheral target) was computed.

5.2.3 Neuropsychological Tasks

Subjects completed the Stockings of Cambridge (SOC) and Spatial Working Memory (SWM) computerized neuropsychological tests from the Cambridge Neuropsychological Test Automated Battery (CANTAB® , Cambridge Cognition, Cambridge, United Kingdom), as previously described in detail (Chapter 3). These tasks probe aspects of planning, strategy use, and spatial working memory.

In the SOC test, the computer screen was divided horizontally into two; the top portion representing the computer's problem set, and the bottom portion the subject's problem set. The computer created a problem set in the top portion of the screen and subjects were then asked to copy the same configuration by moving coloured balls on the computer screen so that the two problem sets were identical. As the minimum number of moves increased, the complexity of the problems increased as well. Outcome measures used for correlational analyses included the total number of problems solved in the minimum number of moves and the initial mean thinking time for 4- and 5-move problems.

In the SWM task, subjects were presented with randomly distributed coloured boxes ranging in number from 4 to 8. Subjects were required to locate hidden tokens that appeared under each coloured box. Once a token was located, the box was recovered and appeared identical to the other boxes. Subjects were informed that the computer would never hide a token in a coloured box previously found to contain one; therefore, they had to recall which boxes they had already searched and *not* revisit them. The outcome measures used for correlational analyses included the total number of errors (returning to

a box previously found to contain a token) and the strategy score, which indicated the use of a search strategy (a low scores indicated good strategy use).

5.2.4 Data Analysis

Direction errors in the antisaccade task were correlated with the neuropsychological task outcome measures, as described above. Each data set was analyzed for normality, and those that did not meet criteria for normal distribution underwent non-parametric analysis using Spearman's correlation. Pearson's correlation was used for parametric data and linear regression analyses were performed when appropriate (Prism v.5.0, GraphPad Inc.). Significant correlations are indicated by $p<0.05$. The correlational figures were further classified by the following age bins: 8-10 years, 11-12 years and 13+ years to determine if any clustering occurred among the different age groups.

5.3 Results

An inverse correlation was found between direction errors in the antisaccade task and initial mean thinking time for 4- and 5-Choice problems in the SOC task for children with FASD (4-Choice: Spearman's $r = -0.34$, $p < 0.01$; 5-Choice: Spearman's $r = -0.29$, $p < 0.05$) (Fig. 5.1). This correlation demonstrated that those children with FASD who made fewer direction errors, also spent more timing planning their strategy for solving both the 4- and 5-Choice problems, as indicated by the increased mean initial thinking time. The same correlation was not apparent for control subjects. An inverse correlation also was evident for children with FASD when the direction errors in the antisaccade task and the total number of problems solved in the minimum number of moves in the SOC task were contrasted (Spearman $r = -0.31$, $p < 0.01$). This correlation revealed that the children, who made fewer direction errors, were the same children that solved more problems in the SOC task (data not shown).

Direction errors in the antisaccade task also were correlated with errors and strategy score outcome measures in the SWM task (Fig. 5.2). Both control and children with FASD demonstrated a positive correlation between the number of direction errors in the antisaccade task and the number of errors committed in the SWM task (FASD: Pearson's $r = 0.39$, $p < 0.001$; Control: Pearson's $r = 0.26$, $p < 0.05$) (Fig. 5.2A,B). The correlation indicated that children who committed fewer direction errors in the antisaccade task also generated fewer errors in the SWM task. From the figure, it appeared that the children in the control group clustered according to their age group, such that the youngest children (8-10 years) demonstrated the poorest performance, while the older children (13+ years) committed fewer direction errors in the antisaccade task

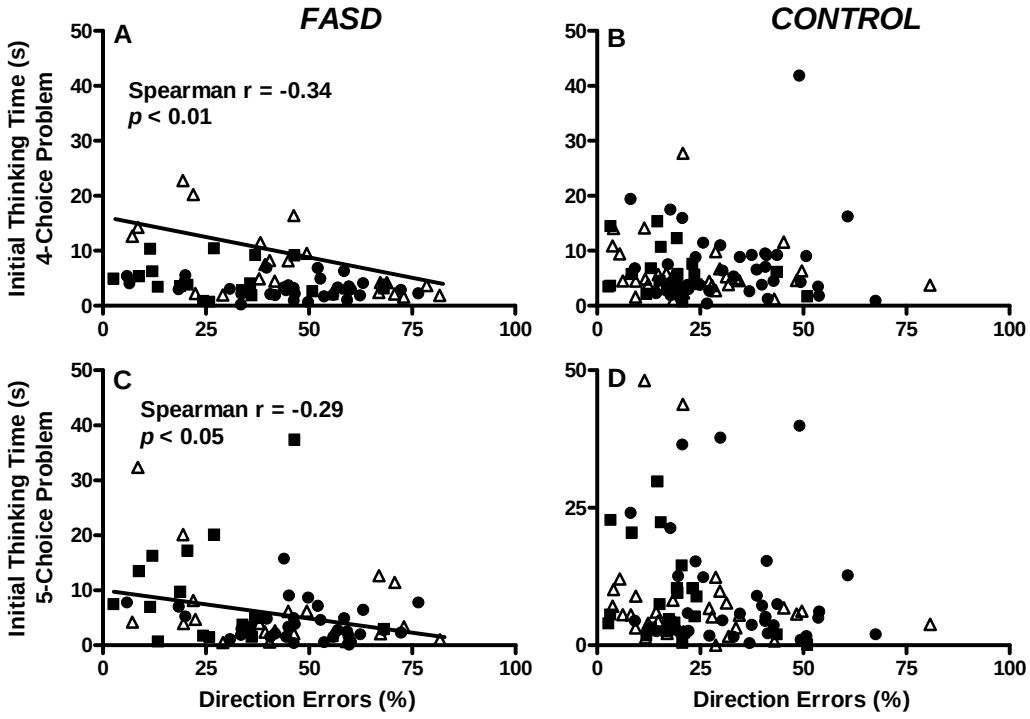

Figure 5.1 Correlations for the initial thinking time for 4-choice (A,B) and 5-choice (C,D) problems in the SOC task and percentage of direction errors in the antisaccade task for subjects with FASD (A,C) and controls (B,D). Filled circles, 8-10 years; open triangles, 11-12 years; filled squares, 13+ years. Linear regressions appear when correlations were significant ($p < 0.05$).

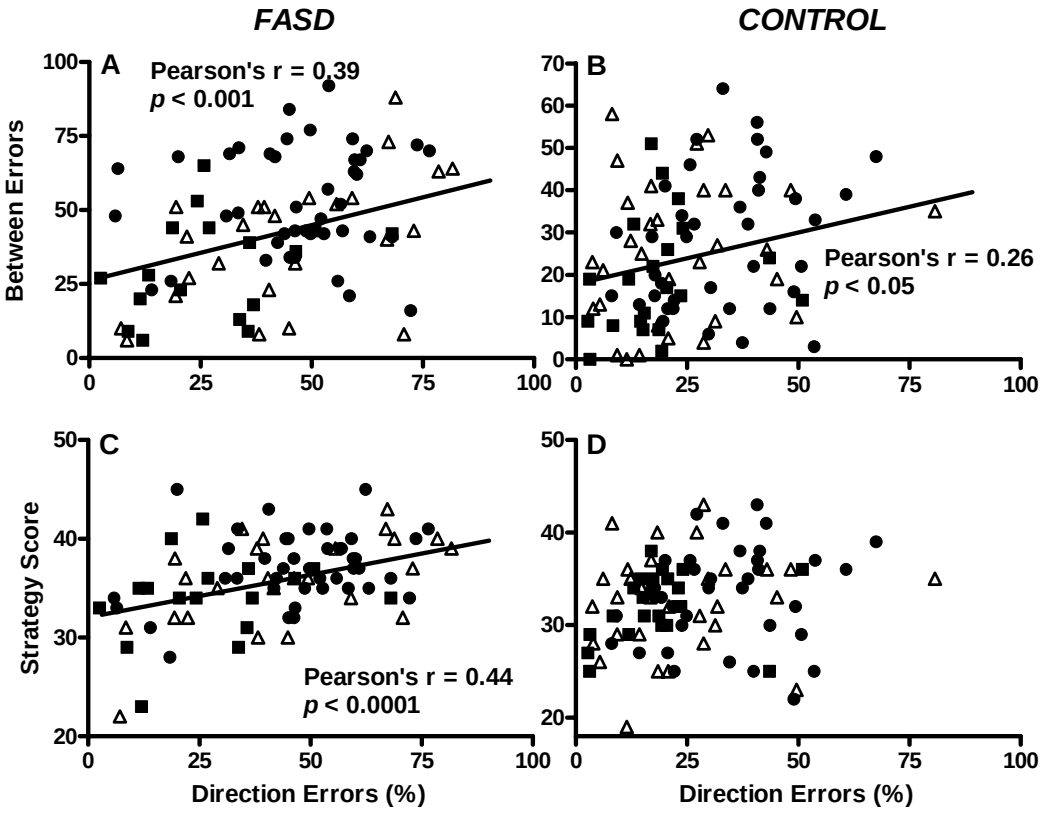

Figure 5.2 Correlations for errors (A,B) and strategy score (C,D) in the SWM task and percentage of direction errors in the antisaccade task for subjects with FASD (A,C) and controls (B,D). Filled circles, 8-10 years; open triangles, 11-12 years; filled squares, 13+ years. Linear regressions appear when correlations were significant ($p < 0.05$).

and errors in the SWM task. The same clustering of age groups was not apparent among subjects with FASD, though there was no age by group interaction.

Finally, a significant positive correlation between direction errors in the antisaccade task and strategy score in the SWM task was apparent for children with FASD (Fig. 5.2C). This correlation indicated that the children who committed fewer direction errors also demonstrated better use of a search strategy (Pearson's $r = 0.44$, $p < 0.0001$). This same correlation was not apparent in the control group.

5.4 Discussion

Here, we demonstrate for the first time that oculomotor behaviours correlate with neuropsychological performance on tasks of planning, strategy and spatial working memory in children with FASD, suggesting a relationship between eye movement deficits and poor performance across tasks of neuropsychological function. In addition, the data suggest a developmental pattern among control subjects, in which the younger children demonstrate poorer performance across tasks, while the oldest children have fewer difficulties; and the same clustering was not always apparent for children in the FASD group. This observation suggests that performance deficits in the children with FASD cannot be explained simply by developmental delay. We now review the oculomotor neurocircuitry and then relevant findings will be discussed as they relate to function and brain activity.

5.4.1 The antisaccade

Oculomotor tasks are an ideal tool for assessing executive function in children (Salman et al., 2006;Munoz et al., 1998) and these tasks have been used to elucidate the neural mechanisms involved in oculomotor control using both non-human and human subjects (Munoz and Everling, 2004;Schall, 2004;Munoz and Schall, 2004;Everling and Munoz, 2000;Munoz et al., 2000). The antisaccade task requires the recruitment of structures located in the frontal and parietal cortices, basal ganglia and brainstem. These structures have also been implicated in executive processes, and as such the antisaccade task has been used to assess executive function in multiple clinical populations (Munoz et al., 2007;Ramat et al., 2007). The antisaccade task is a two step process that requires subject to 1) inhibit the automatic response to look at the target and 2) generate a

voluntary movement in the opposite direction (Fig. 1.1). The key brain structures responsible for performing antisaccades are reviewed [for more detailed explanation of saccadic eye movement neural circuitry please see (Gaymard et al., 1998;Pierrot-Deseilligny et al., 1995;Munoz and Everling, 2004)].

Two key structures play a critical role in the suppression of the automatic response and the generation of goal-directed saccades: the frontal eye fields (FEF) and the intermediate layers of superior colliculus (SCi) (Munoz and Everling, 2004). Both the FEF and SCi contain distinct populations of *fixation* and *saccade* neurons (Munoz et al., 2000). Fixation neurons are tonically active during fixation and cease to fire during saccadic activity, while saccade neurons are silent during fixation and fire high-frequency action potentials for saccades on the contralateral visual field. For successful antisaccade trials, where the target appears in the right visual field, saccade neurons in the left FEF and SCi must be inhibited, while saccade neurons in the right FEF and SCi become activated to drive the leftward saccade. Direction errors occur when there is insufficient suppression of saccade neurons in the FEF and SCi prior to target appearance. Another important region that contributes to the accumulation of activity toward threshold is the supplementary eye fields (Schlag-Rey et al., 1997), which send motor commands to the brainstem premotor circuit to enhance the motor command from FEF and SCi for the successful production of an antisaccade. A second source of inhibitory signalling arises in the dorsolateral prefrontal cortex (dlPFC), as it projects directly to the SCi and FEF. Lesions to the dlPFC lead to a marked increase in the occurrence of direction errors generated in the antisaccade paradigm (i.e., erroneous automatic prosaccades to the cue), demonstrating a deficit in reflexive saccade inhibition (Pierrot-Deseilligny et al.,

1991;Guitton et al., 1985). As the frontal cortex is associated with successful performance of executive function tasks (Funahashi, 2001), dysfunction to this region likely occurs as a consequence to prenatal alcohol exposure and may underlie the deficits in antisaccade performance observed for children with FASD.

5.4.2 Neuropsychological outcome measures

The SOC task measures spatial planning and behavioural inhibition, and during the task children were encouraged to spend time planning an appropriate strategy for solving each problem set. The negative correlation in mean initial thinking time for 4- and 5-choice problems versus the percentage of direction errors in the FASD group strongly suggests an impairment in the ability to properly plan solutions to a given problem. Alternatively, the decreased time spent planning a strategy could be interpreted as evidence for impulsivity. The negative correlation between initial thinking time for SOC and direction errors in the antisaccade task strongly suggests that dysfunction in regions of the frontal cortex, and possibly parietal cortex, underlie many of the observations revealed for children with FASD.

In the SWM task, subjects were required to use mnemonic information to work towards a goal, such as devising a search strategy to reduce the memory load for a given trial, by following a predetermined search sequence. The strong positive correlation between direction errors in the antisaccade task and errors in the SWM task for both the FASD and control groups (Fig. 5.2A,B), also suggest the involvement of regions of frontal cortex, consistent with the findings described earlier. Interestingly, by clustering subjects by age bins, it appeared that the younger children were poorer performers in the control group, while the older children were much better. For children with FASD

performance varied across the age bins and could not be attributed to development, suggesting that protracted maturation of the frontal lobes could not fully explain the correlation.

Deficits in working memory are common in individuals with FASD (Rasmussen, 2005), and working memory is a key component of human cognition. Recently, working memory performance was assessed in typically developing children and adolescents (Conklin et al., 2007). The findings revealed an improvement in performance on most working memory tasks that was consistent with development and known brain maturation events. In particular, improvements after age 12 were found in both spatial and verbal domains, but no improvement on recognition memory was found in the study ages. The improvements in the former two domains were supported by the fact that these functions are governed by the frontal lobes, which undergo delayed maturation. On the contrary, the latter function (recognition memory) is governed by posterior substrates, which mature earlier negating further improvements. Taken together, these observations suggest a hierarchical pattern of working memory development. This is consistent with an earlier study that demonstrated that working memory development begins with the refinement of basic perceptual and sensorimotor functions, and concludes with the physiological maturation of widespread neural networks that can perform complex working memory tasks (Luciana and Nelson, 1998). Thus, it is plausible for simple tasks associated with working memory to remain intact in children with FASD, while other functions associated with working memory that require higher cognitive demand, to reveal profound deficiencies.

After assessing children with prenatal alcohol exposure on elements of encoding (working memory), shifting, focused and sustained attention, it was the working memory deficits that were primarily associated with FASD (Burden et al., 2005). In particular, tests that required the active manipulation of information in memory-related task execution were most difficult for these individuals. Consistently, Kodituwakku et al. (1995) also found that based on task demands, children with FASD had greater difficulty performing tests that maintained a high working memory component (Kodituwakku et al., 1995). As working memory involves the ability to hold and manipulate visual-spatial information and the maintenance and rehearsal of verbal information, it may represent a core deficit in FASD. As such, working memory development has been associated with posterior-to-anterior brain development, which suggests that impairments are associated with alterations to this developmental pattern arising from the prenatal alcohol insult. Findings from Conklin et al. (2007) further support this posterior-to-anterior developmental pattern, as they found that working memory performance mediated primarily by the dorsolateral prefrontal cortex was stabilized *after* performance on tasks that were governed by ventral substrates. Thus, it is highly likely that prenatal alcohol exposure has long-lasting effects on brain maturation that occur into postnatal life, well after the initial insult has been made.

5.4.3 Conclusion

Deficits in eye movement control are correlated with cognitive dysfunction in children with FASD, such that prenatal alcohol appears to affect brain development in a long-lasting fashion. In particular, the frontal cortex appears to be particularly sensitive to alcohol teratogenicity and deficient maturation, to both structure and function, is

consistent with the data from this study. Additionally, testing children across multiple sites is now feasible, as the technology is mobile. Thus, eye movement behaviours may be a powerful tool for providing insight into the cognitive deficits, and underlying cortical and subcortical structures, that are most compromised in this population.

Chapter 6 GENERAL DISCUSSION

This study was conducted to explore the feasibility of developing a novel and objective tool for assessing the brain injury resulting from prenatal alcohol exposure. Thus, the aim was three-fold: 1) to demonstrate deficits in oculomotor control in children with FASD, 2) to measure deficiencies in executive function using standardized computer based testing, and 3) to adapt these research tools for mobile use in community settings across multiple test sites. The general hypotheses and major findings from each paper are summarized.

To address the first hypothesis that children with FASD will demonstrate specific deficits in oculomotor control that can be measured using saccadic eye movement tasks, we conducted a preliminary study. In a sample of 10 children with FASD, profound deficits in oculomotor control were demonstrated, providing compelling evidence for further investigation using a larger sample size. This pilot work set the foundation for the multi-centered project that involved 9 communities across Canada.

In the multi-centered study, the increased subject recruitment pool enabled us to address two important questions that could not be tested in the first study. Namely, the effects of age and diagnosis on deficits in eye movement control and performance in standardized neuropsychological tasks was also assessed. We demonstrated again that deficits in saccadic eye movement tasks are associated with brain injury resulting from prenatal exposure to alcohol. The data confirmed that children between the ages of 8-15 years exhibited profound deficits across many outcome measures for both pro- and antisaccade tasks that suggests specific deficits in FEF, SEF and PEF function. We also

found no evidence for developmental delay across the age ranges tested, nor were there substantial differences among the diagnostic subgroups (FAS, pFAS, ARND).

We assessed multiple domains of executive function using standardized neuropsychological tasks to demonstrate whether children with FASD have specific and global deficits. Using the CANTAB® tool, the data revealed profound deficits in attention, planning, strategy and working memory, revealing that the neurocognitive problems associated with FASD were widespread and generalized; and not specific to one particular domain. Finally, we demonstrated a significant correlation between oculomotor deficits and deficiencies in executive function. By correlating behavioural data with neuropsychological outcomes, it was possible to speculate as to the brain regions most sensitive to a prenatal alcohol insult. In particular, it was the frontal lobe dysfunction that appeared to underlie many of the deficits we found in both measures of executive function. With the use of functional MRI studies that assess oculomotor control and performance in neuropsychological tasks, it will be possible to more definitively determine the particular regions of the frontal cortex and/or brainstem that are most sensitive to prenatal alcohol injury.

6.1 Clinical Relevance

The need for standardized, objective tools to assist in the diagnosis of individuals with a history of prenatal alcohol exposure is necessary (Chudley et al., 2005). It is now well appreciated that individuals who receive an early diagnosis demonstrate significantly better outcomes and a reduction in the prevalence of secondary disabilities (Streissguth et al., 2004;Streissguth et al., 1991;Streissguth et al., 1985). Currently, many of the standardized techniques that are available for diagnosis are not appropriate for culturally

diverse populations. Additionally, they may not be suitable for infants and toddlers, who are too young to undergo extensive neuropsychological assessment. Thus, the potential for saccadic eye movement tasks and tests of executive function have significant applicability for use as potential screening tools to assist in the early diagnosis of alcohol-related disabilities.

Recently, a rural FASD diagnostic service model has been implemented in Cold Lake, Alberta, Canada (McFarlane and Rajani, 2007). The Lakeland Centre provides services to the region's communities through mobile diagnostic teams and follow-up support personnel. It was established in 1996 using the 4-Digit Code system, but adapted to the Canadian diagnostic guidelines in 2005. It operates three multidisciplinary, diagnostic teams (two for children, one for adults) that consist of a pediatrician, neuropsychologist, speech-language pathologist, occupational therapist, public health nurse, Aboriginal liaison, mental health therapist, social worker, addictions counsellor and team coordinator. The child teams diagnose approximately 4 children per month; while the adult team diagnose 1 adult per month. The diagnostic process consists of a pre-clinic phase (Phase 0) and 5 clinical phases: assessments, discussion of results, client debriefing, write up and team debriefing and post clinic. The model was adapted for rural diagnosis, and includes in-kind donations, follow-up support and mobile teams. Such a model may provide the framework for other diagnostic service systems both in and outside of Canada. Tools such as the ones described in this research project could be easily adapted for use in these mobile diagnostic clinics.

This study demonstrates that oculomotor tasks and standard neuropsychological tests are objective, sensitive measures of global brain injury associated with prenatal

alcohol exposure. Future research must be conducted to assess whether these tools can be used to assess brain function in younger children, and to improve upon the existing protocols to probe more specific aspects of behaviour. Although, the sensitivity and specificity of these tools remains to be determined, this study provides significant support for their clinical applicability. Eye movement and standardized neuropsychological testing tools hold significant promise for assisting in the screening and diagnosis of individuals with a history of prenatal alcohol exposure.

6.2 Future Directions

1. Based on performance of oculomotor tasks, it appeared that children with FASD have specific deficits in frontal lobe and brainstem function, in particular the FEF, dlPFC and SC. To further support or refute this claim, brain function must be assessed using the pro- and antisaccade tasks with function magnetic resonance imaging. Imaging studies can confirm the extent of damage to these regions, as a result of a prenatal alcohol insult. Furthermore, these studies can be further stratified by age, sex and diagnostic subtype (i.e., FAS, pFAS or ARND) to provide further insight into the specific types of damage, and how these covariates influence brain function.
2. While evidence for developmental delay was not found in our study, future investigations involving young adults (i.e., 18-24 years) would further support this finding. In young adults, frontal lobe maturation is nearing completion (or complete). By studying this age range, it would be possible to determine if performance becomes comparable to controls, or whether performance deficits persist. If these individuals continue to demonstrate profound deficits in

oculomotor control and neuropsychological function, the pathophysiology underlying these deficits cannot be attributed to developmental delay (i.e., protracted maturation of frontal lobes), and instead suggest other mechanisms. This insight may assist in the development of novel programs and intervention strategies aimed at improving overall brain function for individuals with neurological injury resulting from prenatal exposure to alcohol.

3. With early diagnosis and access to supports, the likelihood of developing secondary disabilities for individuals with FASD decreases profoundly (Streissguth et al., 2004;Streissguth et al., 1991;Streissguth et al., 1985). Thus, oculomotor tasks that can be used to assess children, who are younger than 8 years of age, would be beneficial. Experiments that could use head mount-free systems would be well suited for younger populations, in which calibration time is reduced. As this study demonstrated profound deficits even in the prosaccade task, it is plausible that future experiments could incorporate simple eye movement protocols that could still be used to assess neurological function. For example, experiments involving movie clips could be conducted in young children, and these tracings could be analyzed off-line for differences in performance.
4. Both animal and human studies have demonstrated hippocampal damage as a result of prenatal alcohol exposure (Mattson et al., 2001) and hippocampal function can be probed using oculomotor tasks (Pierrot-Deseilligny et al., 1995). In particular, memory-guided saccade tasks would be a suitable protocol for assessing working memory in individuals with FASD. In a version of this task,

subjects are seated in front of computer or projection screen and after a brief delay; three target lights appear at random locations one after another. After another brief delay, subjects must recall the location and pattern of the target lights by moving their eyes in the same pattern. Preliminary data have been collected for control children in the 8-12 year range, thus these children are capable of performing the task.

5. Using the data sets collected in this study, it may be possible to generate normative data sets for children with FASD based on the oculomotor and neuropsychological outcome measures. To assess the specificity and sensitivity of these tools, prospective testing could be conducted in the existing diagnostic clinics. Once the data sets are obtained, they could be compared to the normative data sets generated from this study. This would further assess the validity of these tools for both screening and diagnostic purposes.
6. Finally, during the course of this study, 11 children with a diagnosis of attention-deficit hyperactivity disorder (ADHD) were also tested with the future goal of comparing data sets between ADHD, FASD and controls. With the recruitment of additional 15-20 subjects with ADHD, it would be possible to conduct a 3-way comparison study (by matching the ADHD children to a subset of FASD and controls by age and sex). As ADHD and FASD are often misdiagnosed (Coles et al., 1997;Nash et al., 2006), this study may provide insight into differences in function among these clinical populations. These differences may be exploited for differential diagnostic purposes that could be used to further increase the specificity and sensitivity of existing diagnostic tools.

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Appendix A: Institute of Medicine: Diagnostic Criteria for Fetal Alcohol Syndrome (FAS) and Alcohol-Related Effects (Stratton et al., 1996).

Fetal Alcohol Syndrome

1. FAS with confirmed maternal alcohol exposure

A. Confirmed maternal alcohol exposure
B. Evidence of a characteristic pattern of facial anomalies that includes features such as short palpebral fissures and abnormalities in the premaxillary zone (e.g., flat upper lip, flattened philtrum, and flat midface)
C. Evidence of growth retardation, as in at least one of the following:
    - low birth weight for gestational age
    - decelerating weight over time not due to nutrition
    - disproportional low weight to height
D. Evidence of CNS neurodevelopmental abnormalities, as in at least one of the following:
    - decreased cranial size at birth
    - structural brain abnormalities (e.g., microcephaly, partial or complete agenesis of the corpus callosum, cerebellar hypoplasia)
    - neurological hard or soft signs (as age appropriate), such as impaired fine motor skills, neurosensory hearing loss, poor tandem gait, poor eye-hand coordination

2. FAS without confirmed maternal alcohol exposure

B, C, and D as above

3. Partial FAS with confirmed maternal alcohol exposure

A. Confirmed maternal alcohol exposure
B. Evidence of some components of the pattern of characteristic facial anomalies

Either C or D or E

C. Evidence of growth retardation, as in at least one of the following:
    - low birth weight for gestational age
    - decelerating weight over time not due to nutrition
    - disproportional low weight to height
D. Evidence of CNS neurodevelopmental abnormalities, as in:
    - decreased cranial size at birth
    - structural brain abnormalities (e.g., microcephaly, partial or complete agenesis of the corpus callosum, cerebellar hypoplasia)
    - neurological hard or soft signs (as age appropriate) such as impaired fine motor skills, neurosensory hearing loss, poor tandem gait, poor eye-hand coordination
E. Evidence of a complex pattern of behavior or cognitive abnormalities that are inconsistent with developmental level and cannot be explained by familial background or environment alone, such as learning difficulties; deficits in school

performance; poor impulse control; problems in social perception; deficits in higher level receptive and expressive language; poor capacity for abstraction or metacognition; specific deficits in mathematical skills; or problems in memory, attention, or judgment

Alcohol-Related Effects

Clinical conditions in which there is a history of maternal alcohol exposure and where clinical or animal research has linked maternal alcohol ingestion to an observed outcome. There are two categories, which may co-occur. If both diagnoses are present, then both diagnoses should be rendered.

4. Alcohol-related birth defects (ARBD)
List of congenital anomalies, including malformations and dysplasias

- Cardiac
  - Atrial septal defects
  - Aberrant great vessels
  - Ventricular septal defects
  - Tetralogy of Fallot
- Skeletal
  - Hypoplastic nails
  - Clinodactyly
  - Shortened fifth digits
  - Pectus excavatum and carinatum
  - Radioulnar synostosis
  - Klippel-Feil syndrome
  - Flexion contractures
  - Hemivertebrae
  - Camptodactyly
  - Scoliosis
- Renal
  - Aplastic, dysplastic,
  - Ureteral duplications
  - Hypoplastic kidneys
  - Hydronephrosis
  - Horseshoe kidneys
- Ocular
  - Strabismus
  - Refractive problems secondary to small globes
  - Retinal vascular anomalies
- Auditory
  - Conductive hearing loss
  - Neurosensory hearing loss
- Other

Virtually every malformation has been described in some patient with FAS. The etiologic specificity of most of these anomalies to alcohol teratogenesis remains uncertain.

5. Alcohol-related neurodevelopmental disorder (ARND)

Presence of:

A. Evidence of CNS neurodevelopmental abnormalities, as in any one of the following:
   - decreased cranial size at birth
   - structural brain abnormalities (e.g., microcephaly, partial or complete agenesis of the corpus callosum, cerebellar hypoplasia)
   - neurological hard or soft signs (as age appropriate), such as impaired fine motor skills, neurosensory hearing loss, poor tandem gait, poor eye-hand coordination

and/or

B. Evidence of a complex pattern of behavior or cognitive abnormalities that are inconsistent with developmental level and cannot be explained by familial background or environment alone, such as learning difficulties; deficits in school performance; poor impulse control; problems in social perception; deficits in higher level receptive and expressive language; poor capacity for abstraction or metacognition; specific deficits in mathematical skills; or problems in memory, attention, or judgment

Appendix B: 4-digit diagnostic code (Astley and Clarren, 2000).

	Growth Deficiency	*FAS facial phenotype*	*Brain Damage*	*Prenatal Alcohol*
4	Significant	Severe	Definite	High risk
3	Moderate	Moderate	Probable	Some risk
2	Mild	Mild	Possible	Unknown
1	None	Absent	Unlikely	No risk

Diagnostic category name and 4-Digit Diagnostic Codes within each category

A Fetal alcohol syndrome (alcohol exposed)

3433 4433 3434 4434 3443 4443 3444 4444

B Fetal alcohol syndrome (alcohol exposure unknown)

3432 4432 3442 4442

C Atypical fetal alcohol syndrome (alcohol exposed)

1443 1434 2434 3334 4334 2443 1444 2444 3344 4344 4343

D Fetal alcohol syndrome phenocopy (no alcohol exposure)

3431 4341 4441 3441 4431

E Sentinel physical findings/static encephalopathy (alcohol exposed)

1333 1433 2344 3143 3243 4133 4233 4333 1334 2333 2433

3144 3244 4134 4234 1343 2334 3133 3233 3333 4143

4243 1344 2343 3134 3234 3343 4144 4244

F Static encephalopathy (alcohol exposed)

1133 1144 1243 2134 2233 2244 1134 1233 1244 2143 2234

1143 1234 2133 2144 2243

G Sentinel physical findings/neurobehavioural disorder (alcohol exposed)

1323 2323 3123 3323 4123 4323 1324 2324 3124 3324 4124

4324 1423 2423 3223 3423 4223 4423 1424 2424 3224 3424

4224 4424

H Neurobehavioural disorder (alcohol exposed)

1123 2123 1124 2124 1223 2223 1224 2224

I	Sentinel physical findings (alcohol exposed) 1313 2313 3113 3313 4113 4313 1314 2314 3114 3314 4114 4314 1413 2413 3213 3413 4213 4413 1414 2414 3214 3414 4214 4414
J	No cognitive/behavioural or sentinel physical findings detected (alcohol exposed) 1113 2113 1114 2114 1213 2213 1214 2214
K	Sentinel physical findings/static encephalopathy (alcohol exposure unknown) 1332 2332 3132 3332 4232 1342 2342 3142 3342 4242 1432 2432 3232 4132 4332 1442 2442 3242 4142 4342
L	Static encephalopathy (alcohol exposure unknown) 1132 1232 2132 2232 1142 1242 2142 2242
M	Sentinel physical findings/neurobehavioural disorder (alcohol exposure unknown) 1322 2322 3122 3322 4122 4322 1422 2422 3222 3422 4222 4422
N	Neurobehavioural disorder (alcohol exposure unknown) 1122 1222 2122 2222
O	Sentinel physical findings (alcohol exposure unknown) 1312 2312 3112 3312 4112 4312 1412 2412 3212 3412 4212 4412
P	No cognitive/behavioural or sentinel physical findings detected (alcohol exposure unknown) 1112 2112 1212 2212
Q	Sentinel physical findings/static encephalopathy (no alcohol exposure) 1331 2341 3231 4141 1341 2431 3241 4231 1431 2441 3331 4241 1441 3131 3341 4331 2331 3141 4131
R	Static encephalopathy (no alcohol exposure) 1131 2131 1141 2141 1231 2231 1241 2241
S	Sentinel physical findings/neurobehavioural disorder (no alcohol exposure) 1321 3121 4121 1421 3221 4221 2321 3321 4321 2421 3421 4421

T	Neurobehavioural disorder (no alcohol exposure) 1121 2121 2221 1221
U	Sentinel physical findings (no alcohol exposure) 1311 3111 4111 1411 3211 4211 2311 3311 4311 2411 3411 4411
V	No cognitive/behavioural or sentinel physical findings detected (no alcohol exposure) 1111 2111 1211 2211

Appendix C: Centers for Disease Control and Prevention. Brief Outline of Diagnostic criteria for Fetal Alcohol Syndrome (Centers for Disease Control and Prevention, 2004).

Facial dysmorphia

Based on racial norms, individual exhibits all three characteristic facial features:

- Smooth philtrum (University of Washington Lip-Philtrum Guide rank 4 or 5)
- Thin vermillion border (University of Washington Lip-Philtrum Guide rank 4 or 5)
- Small palpebral fissures (at or below 10th percentile)

Growth problems

Confirmed prenatal or postnatal height or weight, or both, at or below the 10th percentile, documented at any one point in time (adjusted for age, sex, gestational age, and race or ethnicity).

Central Nervous System Abnormalities

I. Structural

1. Head circumference (OFC) at or below the 10th percentile adjusted for age and sex.
2. Clinically significant brain abnormalities observable through imaging.

II. Neurological

Neurological problems not due to a postnatal insult or fever, or other soft neurological signs outside normal limits.

III. Functional

Performance substantially below that expected for an individual's age, schooling, or circumstances, as evidenced by:

1. Global cognitive or intellectual deficits representing multiple domains of deficit (or significant developmental delay in younger children) with performance below the 3rd percentile (2 standard deviations below the mean for standardized testing) *or*
2. Functional deficits below the 16th percentile (1 standard deviation below the mean for standardized testing) in at least three of the following domains:

   a) cognitive or developmental deficits or discrepancies
   b) executive functioning deficits
   c) motor functioning delays
   d) problems with attention or hyperactivity
   e) social skills
   f) other, such as sensory problems, pragmatic language problems, memory deficits, etc.

Maternal Alcohol Exposure

I. Confirmed prenatal alcohol exposure
II. Unknown prenatal alcohol exposure

Criteria for FAS Diagnosis

Requires all three of the following findings:

1. Documentation of all three facial abnormalities (smooth philtrum, thin vermillion border, and small palpebral fissures);
2. Documentation of growth deficits
3. Documentation of CNS abnormality

Appendix D: Canadian Diagnostic Guidelines for FASD. Harmonization of Institute of Medicine (IOM) nomenclature and 4-digit diagnostic code ranks for growth, face, brain and alcohol history (Chudley et al., 2005)

	4-digit diagnostic code ranks			
IOM Nomenclature	*Growth deficiency*	*FAS facial phenotype*	*CNS damage or dysfunction*	*Gesetational exposure to alcohol*
FAS (with confirmed exposure)	2, 3 or 4	3 or 4	3 or 4	3 or 4
FAS (without confirmed exposure)	2, 3 or 4	3 or 4	3 or 4	2
Partial FAS (with confirmed exposure)	1, 2, 3 or 4	2, 3 or 4	3 or 4	3 or 4
ARND (with confirmed exposure)	1, 2, 3 or 4	1 or 2	3 or 4 (2 for < 6 years)	3 or 4

Appendix E: Clarification of the 1996 IOM Criteria for Diagnosis of FASD (Hoyme et al., 2005).

I. FAS with Confirmed Maternal Alcohol Exposure (requires all features A-D)

A. Confirmed maternal alcohol exposure
B. Evidence of a characteristic pattern of minor facial anomalies, including ≥ 2 of the following:
   - Short palpebral fissures (≤10th percentile)
   - Thin vermilion border of the upper lip (score 4 or 5 with the lip/philtrum guide)
   - Smooth philtrums (score 4 or 5 with the lip/philtrum guide)
C. Evidence of prenatal and/or postnatal growth retardation
   - Height of weight ≤10th percentile, corrected for racial norms, if possible.
D. Evidence of deficient brain growth or abnormal morphogenesis, including ≥1 of the following:
   - Structural brain abnormalities
   - Head circumference ≤10th percentile

II. FAS Without Confirmed Maternal Alcohol Exposure
IB, IC, and ID, as above

III. Partial FAS With Confirmed Maternal Alcohol Exposure (requires all features, A–C)
A. Confirmed maternal alcohol exposure
B. Evidence of a characteristic pattern of minor facial anomalies, including ≥ 2 of the following:
   - Short palpebral fissures (≤10th percentile)
   - Thin vermilion border of the upper lip (score 4 or 5 with the lip/philtrum guide)
   - Smooth philtrum (score 4 or 5 with the lip/philtrum guide)
C. One of the following other characteristics
   - Evidence of prenatal and/or postnatal growth retardation
     - Height or weight ≤10th percentile corrected for racial norms, if possible
   - Evidence of deficient brain growth or abnormal morphogenesis, including ≥1 of the following
     - Structural brain abnormalities
     - Head circumference ≤10th percentile
   - Evidence of a complex pattern of behavioral or cognitive abnormalities inconsistent with developmental level that cannot be explained by genetic predisposition, family background, or environment alone
     - This pattern includes marked impairment in the performance of complex tasks (complex problem solving, planning, judgment,

abstraction, metacognition, and arithmetic tasks); higher-level receptive and expressive language deficits; and disordered behavior (difficulties in personal manner, emotional lability, motor dysfunction, poor academic performance, and deficient social interaction)

IV. Partial FAS Without Confirmed Maternal Alcohol Exposure
IIIB and IIIC, as above

V. ARBD (requires all features, A–C)

A. Confirmed maternal alcohol exposure
B. Evidence of a characteristic pattern of minor facial anomalies, including ≥ 2 of the following
   - Short palpebral fissures (≤10th percentile)
   - Thin vermilion border of the upper lip (score 4 or 5 with the lip/philtrum guide)
   - Smooth philtrum (score 4 or 5 with the lip/philtrum guide)
C. Congenital structural defects in ≥1 of the following categories, including malformations and dysplasias (if the patient displays minor anomalies only, ≥2 must be present):
   - *cardiac*: atrial septal defects, aberrant great vessels, ventricular septal defects, conotruncal heart defects;
   - *skeletal*: radioulnar synostosis, vertebral segmentation defects, large joint contractures, scoliosis;
   - *renal*: aplastic/hypoplastic/dysplastic kidneys, "horseshoe" kidneys/ureteral duplications; *eyes*: strabismus, ptosis, retinal vascular anomalies, optic nerve hypoplasia;
   - *ears*: conductive hearing loss, neurosensory hearing loss;
   - *minor anomalies*: hypoplastic nails, short fifth digits, clinodactyly of fifth fingers, pectus carinatum/excavatum, camptodactyly, "hockey stick" palmar creases, refractive errors, "railroad track" ears

VI. ARND (requires both A and B)

A. Confirmed maternal alcohol exposure
B. At least 1 of the following
   - Evidence of deficient brain growth or abnormal morphogenesis, including ≥1 of the following
     - Structural brain abnormalities
     - Head circumference ≤10th percentile
   - Evidence of a complex pattern of behavioral or cognitive abnormalities inconsistent with developmental level that cannot be explained by genetic predisposition, family background, or environment alone.
     - This pattern includes marked impairment in the performance of complex tasks (complex problem solving, planning, judgment, abstraction, metacognition, and arithmetic tasks); higher-level

receptive and expressive language deficits; and disordered behavior (difficulties in personal manner, emotional lability, motor dysfunction, poor academic performance, and deficient social interaction)

Appendix F: Demographics, Connors Rating Scale and Questionnaire.

F.1 Demographics

Subject enrolment and demographic data was obtained from each primary caregiver and summarized in Table F.1.

F.2 Connors Rating Scale

The Connors' Rating Scale is a commercially available tool (Multi-Health Systems Inc., Toronto, ON, Canada) that uses observer ratings to evaluate problem behaviours in children and adolescents. It was developed by Dr. Keith Connors and has been used in routine screening for attention-deficit hyperactivity disorder. Primary caregivers completed the one-page questionnaire, which provided standardized age- and sex-adjusted T-scores across 6 behavioural domains (Conduct Disorder, Learning Problems, Psychosomatic, Impulsive-Hyperactive, Anxiety and Hyperactivity Index). The CRS–R scales were standardized using a large normative database compiled from over 200 data collection sites throughout North America. Separate norms for boys and girls are provided in 3-year intervals for ages 3 through 17.

Children with FASD demonstrated significantly greater T-scores compared with control subjects in each of the behavioural domains (Fig. F.1A). Figure F.1B depicts T-score breakdowns according to the diagnostic subgroups (i.e., ARND, pFAS, FAS) for children with FASD. There were no differences in scores on the Connors' Rating Scale between the diagnostic subgroups.

F.3 Questionnaire

An extensive questionnaire was created in-house and completed by primary caregivers to provide a comprehensive overview of the education, medical and family

Table F.1. Demographic data for subjects.

Category	Control (n=92)	FASD (n=89)
Age (years)	11.2 ± 0.2	10.7 ± 0.2
Male: Female	40:52	44:45
Parent/Caregiver Level of Education (years)	16.5 ± 0.3	14.4 ± 0.3*
Medication		
Stimulant	0 (0%)	38 (43%)
Antipsychotic	0 (0%)	29 (33%)
Antidepressants	0 (0%)	10 (11%)
Anticonvulsant	0 (0%)	3 (3%)
Other[a]	12 (13%)	20 (22%)
Co-morbidity (≥ 10% of subjects with FASD)		
Sleeping Disorders	10 (11%)	55 (62%)
ADHD/ADD	0 (0%)	53 (60%)
Oppositional Defiant Disorder	0 (0%)	19 (21%)
Anxiety	0 (0%)	15 (17%)
Asthma	12 (13%)	10 (11%)
Depression	1 (1%)	10 (11%)
Ratio of Adults to Children (Home)	0.88 ± 0.04	0.76 ± 0.06*
Living with Biological Parents	87 (97%)	15 (17%)
Parent/Caregiver Employed	79 (88%)	65 (74%)

[a]Antihistamine, Anti-asthma, Oral contraceptives, Melanin

*$p < 0.05$

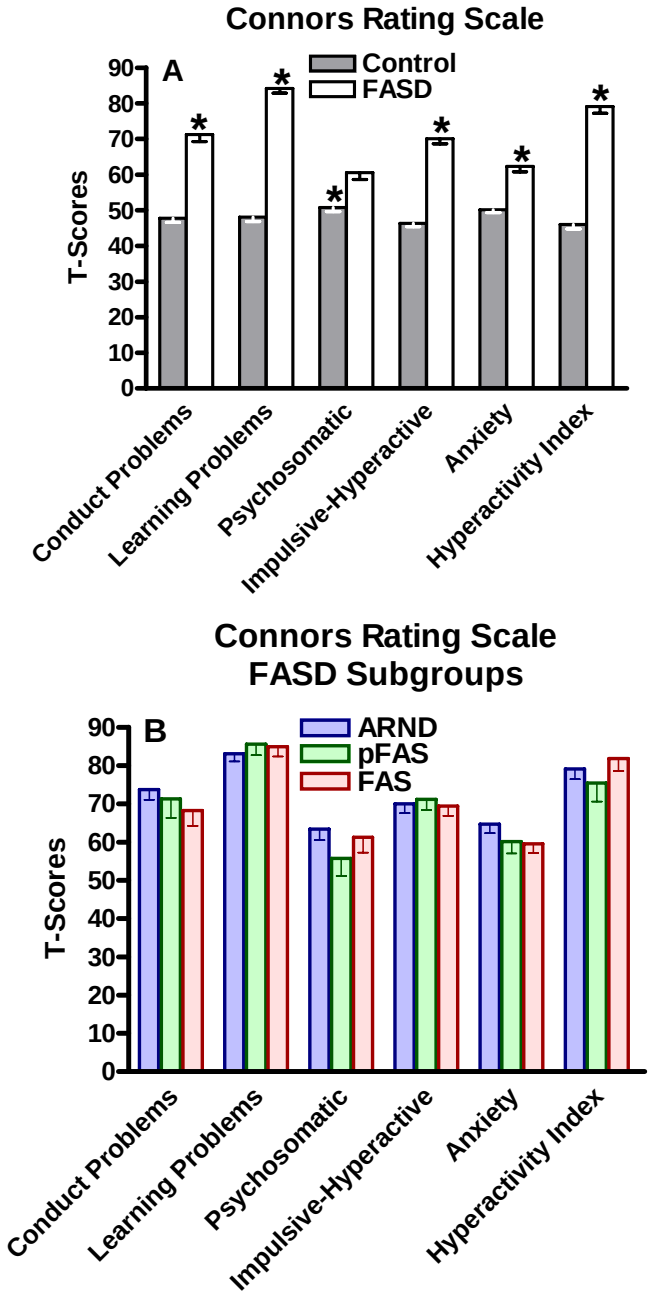

Fig. F.1 a) T-scores for Connors Rating Scale for control children (gray bars) and children with FASD (open bars); and b) T-scores for FASD subgroups, alcohol –related neurodevelopmental disorder (ARND): blue gray bars; partial fetal alcohol syndrome (pFAS): green bars; fetal alcohol syndrome (FAS): red bars, across six behavioural parameters. Values are presented as mean ± S.E.M.

history for each subject. This information was used to determine the type and frequency of co-morbidities (Table 4.1) and medication (Table 4.2) among the children with FASD and control subjects. Interestingly, sleeping disorders was the most commonly reported co-morbidity among children with FASD occurring in 61.7% of the children, while attention-deficit hyperactivity disorder was the second most common in 59.6% of the children with FASD. Stimulants (42.7%) and antipsychotics (32.6%) were the two most commonly reported medications for children with FASD.