Cortical Morphology in Children with Alcohol-Related Neurodevelopmental Disorder

by

Meghna Rajaprakash

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Institute of Medical Science
University of Toronto

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Individuals exposed to alcohol in utero have reduced cortical grey matter volumes. However, the underlying determinants of these reductions have not been investigated exclusively in alcohol-related neurodevelopmental disorder (ARND). Using magnetic resonance imaging scans from 121 participants (57 ARND and 64 controls) aged 8 to 16 years, cortical morphology was analyzed. Results revealed the ARND group had reduced cortical grey matter volumes, but did not differ from controls in cortical thickness. Rather, the cortical abnormalities reflected reductions in global surface area, local surface area reductions in the right occipital-temporal area and right superior temporal gyrus, as well as reduced gyrification. A significant interaction between sex and group was observed, with females showing greater reductions than males in cortical volume and surface area. Results suggest that ARND is characterized by global reductions in cortical surface area and gyrification and females are more vulnerable than males to the teratogenic effects of alcohol.
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**Abbreviations**

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<tr>
<td>ADHD</td>
<td>attention-deficit/hyperactivity disorder</td>
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<td>ARND</td>
<td>alcohol-related neurodevelopmental disorder</td>
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<td>CAS</td>
<td>Children’s Aid Society</td>
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<td>CIVET</td>
<td>corticometric iterative vertex-based estimation of thickness</td>
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<td>CLASP</td>
<td>constrained laplacian-based automated surface-extraction</td>
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<td>CNS</td>
<td>central nervous system</td>
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<td>CSF</td>
<td>cerebrospinal fluid</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>FAE</td>
<td>fetal alcohol effects</td>
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<td>FAS</td>
<td>fetal alcohol syndrome</td>
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<td>FASD</td>
<td>fetal alcohol spectrum disorder</td>
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<td>FDR</td>
<td>False Discovery Rate</td>
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<td>G1</td>
<td>first gap phase</td>
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<td>GI</td>
<td>gyrification index</td>
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<td>HPE</td>
<td>holoprosencephaly</td>
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<td>IOM</td>
<td>Institute of Medicine</td>
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<td>Term</td>
<td>Description</td>
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<td>IQ</td>
<td>intelligence quotient</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<td>MRM</td>
<td>magnetic resonance microscopy</td>
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<tr>
<td>NMDA</td>
<td>N-Methyl-D-aspartate</td>
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<tr>
<td>PAE</td>
<td>prenatal alcohol exposure</td>
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<tr>
<td>pFAS</td>
<td>partial fetal alcohol syndrome</td>
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<td>SES</td>
<td>socioeconomic status</td>
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<td>WASI</td>
<td>Wechsler Abbreviated Scale of Intelligence</td>
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Chapter 1
Introduction

The teratogenic effects of alcohol on the brain are well documented in the condition known as Fetal Alcohol Spectrum Disorders (FASD), which encompasses a range of conditions arising from prenatal exposure to alcohol. These include fetal alcohol syndrome (FAS) and alcohol-related neurodevelopmental disorder (ARND), as well as partial FAS and alcohol-related birth disorders. Among the two most commonly known conditions, FAS and ARND, children diagnosed with FAS show facial dysmorphology, growth deficiencies, and neurobehavioral deficits while children with ARND display only neurobehavioural deficits and the associated physical features are absent (Chudley et al., 2005).

Knowledge of the consequences of maternal drinking during pregnancy has grown exponentially in recent years and it is now recognized to be prevalent in many countries and a leading cause of preventable developmental disabilities and mental retardation. In the United States, for example, the prevalence estimates for FAS range from 0.5 to 7.0 cases per 1000 births (May et al., 2009), while in Canada, this is estimated at 4 in a 1000 births (Chudley et al., 2005). However, the more common form of the condition, ARND, is thought to affect about 1% of births in the United States (May et al., 2009) and 2% in Canada, while rates in Europe may be as high as 4% (May et al., 2006). Unfortunately, however, these figures may underestimate the true prevalence of FASD since many individuals with ARND are undiagnosed or misdiagnosed (Chudley et al., 2007) because they lack the gross physical dysmorphologies necessary for clinical recognition. As such, further research is still needed to identify the biomarkers of ARND, thus allowing for detection of the condition.
Although the neurobehavioral profile of FASD varies across the spectrum and even among individuals within the specific diagnostic categories, all affected individuals display a varied profile of severe and debilitating cognitive deficits. These include both global intellectual deficits as well as specific problems in domains such as executive functioning, social cognition, attention, language, learning and memory, and visuospatial skills (Kodituwakku, 2007). In contrast, simple visual perception is relatively spared in individuals with FASD (Uecker and Nadel, 1996).

Recent brain imaging studies have served to identify the neural correlates of cognitive and behavioural deficits in FASD, particularly in terms of their link to reduced grey matter volumes within the cortex (for a review, see Lebel et al, 2011). However, the underlying determinants of such cortical reductions, namely thickness and surface area and its associated feature gyrification, have not been fully elucidated in FASD subgroups. It is also unknown whether atypical cortical features underlie the clinical and neurobehavioural manifestations among individuals with specifically the ARND (invisible) subtype.

Thus, the purpose of the current thesis will be to investigate the nature of cortical abnormalities in these individuals who represent a relatively understudied subset of the FASD population as compared with the FAS subtype. To provide a clinical context for ARND, I will first give an overview of the various FASD diagnostic classifications systems. I will next summarize the mechanisms of alcohol teratogenicity on the developing brain reviewing both the relevant animal literature and human brain imaging studies on prenatal alcohol exposure. Finally, I will discuss at length the determinants of normative cortical development and the recent techniques and research that have helped elucidate alcohol’s effect on the developing cortex.
Chapter 2
Literature Review

2.1 Clinical Context

2.1.1 History of Diagnosis in FASD

The 1968 study by Lemoine et al. from France was the first to describe the particular pattern of birth defects associated with prenatal alcohol exposure. These researchers described findings on 127 children of alcoholic mothers (Lemoine et al., 1968), who all reportedly had growth deficiencies, behavioural problems and physical abnormalities, including peculiar facial features. Several years later in the United States, Jones and Smith (1973) coined the term Fetal Alcohol Syndrome (FAS), which involved the following triad of symptoms: a dysmorphic face, growth deficiencies including reduced head size, and central nervous system and neurobehavioural deficits (Jones and Smith, 1973). The particular pattern of facial abnormality in these children included a smooth philtrum (region between the upper lip and nose), a thin vermillion border (exposed upper lip), and short palpebral fissure (distance between the inner and outer canthus of the eye), and other dysmorphologies such as epicanthal folds and a low nasal bridge.

Importantly, these seminal observations on patients who were fetal alcohol affected sparked a surge of animal studies, case reports of FAS, and large-scale epidemiological studies of pregnant women. The latter included four prospective studies conducted in Seattle, Pittsburgh, Atlanta, and Detroit on large samples of women whose drinking histories were recorded and related to later consequences in the offspring. The 1974 Seattle Prospective Longitudinal Study on Alcohol and Pregnancy identified and followed 500 mother-child
pairs at regular intervals over many years. This study was especially significant in that it used a quantity-frequency-variability approach to delineate the relationship between nature of alcohol exposure and outcomes across the first 25 years of development. The initial results of this study confirmed that children with high prenatal alcohol exposure had worse outcomes than did those whose mothers drank minimally or not at all. The former patients not only were smaller in size and had neurological impairments, reduced brain volumes, and physical dysmorphologies (Steissguth et al., 1981), but they also had lower IQs and performed more poorly on a number of outcome measures including attention and processing speed (Streissguth et al., 1984; Streissguth et al., 1989). As adults, affected individuals were at high risk of mental illness and other secondary disabilities (Streissguth et al., 1996).

These early studies led to a common understanding that prenatal alcohol exposure results in a broad and varying spectrum of physical abnormalities and neurobehavioural deficits that vary in degree of severity. Many of the children who did not meet full criteria for an FAS diagnosis were still adversely affected in terms of neurobehavioural deficits. For this reason, a new term derived from the animal literature, known as “FAE” (i.e., fetal alcohol effects) was used to denote the outcomes that deviate from the criteria for an FAS diagnosis (Clarren and Smith, 1978). However, the term “FAE” was later abandoned for a more precise system of classification (Sokol and Clarren, 1989).

As a first step in developing a common diagnostic system, the Institute of Medicine (IOM) in the United States proposed a classification scheme with five major diagnostic categories. These included: (i) FAS with confirmed maternal alcohol exposure; (ii) FAS without confirmed maternal alcohol exposure (iii) partial FAS for those who show some physical anomalies; (iv) alcohol-related birth defects for those who show physical abnormalities only,
and (v) alcohol-related neurodevelopmental disorder (ARND, Stratton et al., 1996; Hoyme et al., 2005). Of particular significance was the creation of the new ARND diagnostic group, which is the most prevalent FASD subtype and is composed of individuals who do not show the evident physical abnormalities. Rather, individuals diagnosed with ARND show central nervous system and neurobehavioural deficits that cannot be explained by genetic factors. Although the IOM system was successful in broadly characterizing the various sub-conditions within the FASD spectrum, it was greatly limited in terms of diagnostic specificity. In particular, the IOM system only provided diagnostic guidelines and lacked an objective scale for measuring the number and severity of presenting deficits (Astley and Clarren, 2000).

To improve on the IOM system, Astley and Clarren (2000) from Seattle developed the 4-Digit Diagnostic Code, which measured symptom severity in three domains, namely growth, face, and central nervous system (CNS) and also assessed the amount of prenatal exposure as a fourth category (see Table 1). For each of these categories (growth, face, CNS, exposure), a score of 1 to 4 is assigned, with a “1” signifying the absence of a particular feature and a “4” reflecting its complete manifestation. From the 256 possible combinations of scores on the four factors, 22 were considered diagnostic by Astley and Clarren (2000) and worthy of further services. This 4-digit system therefore allowed for greater precision in classifying FASD and to this day, is the one most widely used in research and clinical practice, especially as it allows insurance companies a means of determining coverage for services. Unfortunately, this system is imprecise in assigning values to specific neurobehavioural deficits in fetal alcohol affected individuals. As such, a third Canadian system was developed to describe the neurobehavioural sequelae more accurately.
2.1.2 The Canadian System of Screening and Diagnosis

The Canadian System of Diagnosis (Chudley at al., 2005) offers a more sensitive way of rating deficits as compared to the two aforementioned approaches. This system incorporates the diagnostic categories of the IOM system with the precision of the scale system within 4-Digit Code (see Table 1.) It also offers stricter guidelines for evaluating brain dysfunction.

According to the Canadian guidelines, children are referred to an FASD clinic if they present with cognitive or behavioural problems suspected to be due to prenatal alcohol exposure. The mother’s history of heavy alcohol consumption must be substantiated by maternal verification, medical records reporting positive blood alcohol, mother receiving alcohol treatment, or reports of other social, legal, and medical problems during pregnancy. If heavy prenatal alcohol exposure is validated, the children undergo a detailed assessment of physical and psychological features at a dedicated clinic, which typically includes a multi-disciplinary team trained in FASD diagnosis. The assessment involves measurements of growth, head size, and facial features, as well as tests of neurobehavioural functioning. To receive an FAS diagnosis, patients must exhibit (a) three facial abnormalities, including short palpebral fissures at or below the 3rd percentile (2 standard deviations below the mean), smooth philtrum and thin vermilion border of the upper lip (score of 4 or 5 on a 5-point Likert scale of the lip-philtrum guide), (b) growth retardation at or below the 10th percentile, and (c) neurobehavioural deficits in 3 domains. The domains examined include: hard and soft neurological signs (e.g. sensory motor), brain structure (e.g. reduced occipitofrontal circumference two or more standard deviations below the mean or microcephaly, abnormal magnetic resonance imaging findings, etc.), general IQ, receptive and expressive communication, academic achievement, memory, executive functioning and abstract
reasoning, attention, social skills, and social communication (Chudley et al., 2005). If the physical abnormalities are absent, patients in Canada may be diagnosed with ARND, provided they show functional deficits as indicated by scoring two standard deviations below the mean in three neurobehavioural domains (see Table 1).

The neurobehavioural assessment includes both simple and complex tasks across a broad range of domains. Individuals are considered “impaired” within a domain if (i) scores on relevant tasks/ measures are 2 or more standard deviations below the mean, (ii) subdomains (e.g. expressive vs. receptive language) are discrepant by at least one 1 standard deviation, or (iii) subtests of a measure are discrepant by at least 1.5-2 standard deviations when considering the reliability and normal variation of the measure. In cases where standardized measurement techniques are unavailable, clinical judgment is relied upon to determine “significant dysfunction” in consideration of the child’s age, socioeconomic status, mental health, and disrupted home and family environments.

Table 1 shows a comparison of the guidelines used in the various systems to define facial abnormalities, growth deficiencies, and neurobehavioural deficits for FAS and ARND subgroups. While all systems provide a means for differentiating between the various FASD subgroups, the 4-Digit Diagnostic Code and Canadian System offer more precise definitions of facial features and growth abnormalities than the IOM system. The Canadian System also provides more specific definitions of neurobehavioural deficit domains.
### Table 1 Differences in diagnostic systems

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<th><strong>4-Digit Diagnostic Code</strong></th>
<th><strong>Canadian System</strong></th>
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<tr>
<td><strong>FAS</strong></td>
<td>Evidence of characteristic facial features</td>
<td>Presence of 3 features: short palpebral fissures (≤ 2 SDs), thin vermillion and smooth philtrum</td>
<td>Presence of 3 features: short palpebral fissures (≤ 2 SDs), thin vermillion and smooth philtrum</td>
</tr>
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<td><strong>Facial Abnormalities</strong></td>
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<tr>
<td><strong>Growth Deficiencies</strong></td>
<td>One of low birth weight for gestational age, decelerating weight over time not due to nutrition, or disproportional low height-to-weight ratio</td>
<td>Height or weight ≤ 10&lt;sup&gt;th&lt;/sup&gt; percentile</td>
<td>Height, weight, or height-to-weight ratio ≤ 10&lt;sup&gt;th&lt;/sup&gt; percentile</td>
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<tr>
<td><strong>Neurobehavioural Deficits</strong></td>
<td>One of decreased cranial size at birth, structural brain abnormalities, or neurological hard or soft signs</td>
<td>Head circumference (≤ 2 SDs), structural, neurological evidence, or dysfunction across three domains (≤ 2 SDs)</td>
<td>3 or more impairments in: hard and soft neurological signs, brain structure, general IQ, communication, academic achievement, memory, executive functioning, attention, social skills, and social communication</td>
</tr>
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</table>

**ARND**

| **Facial Abnormalities** | None | None | None |
| **Growth Deficiencies** | None | None | None |
| **Neurobehavioural Deficits** | Same as FAS | Same as FAS | Same as FAS |
2.1.3 Importance of Early Diagnosis

It is well established that prenatal alcohol exposure leads to a range of primary deficits, which include low IQ, executive functioning, motor skills, communication, learning and memory, attention/hyperactivity, visuospatial skills (Kodituwakku, 2007). Moreover, these primary deficits are precursors of a number of secondary disabilities that arise later in life in most affected individuals (Kodituwakku, 2007). For example, over 90% of adults with FASD suffer from mental illness while the majority are unemployed (80%), have disrupted school experience (60%), exhibit inappropriate sexual behavior (50%), and are likely to be alcoholics themselves (30%) (Streissguth et al., 1996). Suicide is very common (O’Malley and Huggins, 2005) as is criminal behavior and trouble with the law (Streissguth and O’Malley, 2000).

In terms of these secondary disabilities, one surprising finding is that individuals with FAS in fact fare better than do those who do not meet the full criteria for full-blown FAS such as those with ARND (Streissguth et al., 1996). This finding may be because children with FAS are readily eligible for early intervention services, whereas those who do not show physical abnormalities but exhibit neurobehavioural deficits do not have the same access. Indeed, Streissguth (1997) reported that those who were given an accurate diagnosis before the age of 6 years of age were far less likely to develop the secondary disabilities. Factors such as living in a stable nurturing home without exposure to violence and receiving the basic needs, as well as accessing developmental disabilities services were also associated with minimized development of complications later in life (Streissguth et al., 1996). These findings therefore suggest that early and accurate diagnosis is critical to protect against the emergence of secondary disabilities in individuals affected with FASD, especially those with ARND.
However, to date, the specificity of diagnostic criteria for those who lack the physical dysmorphologies remains questionable (Chudley et al., 2007).

2.1.4 Challenges of Diagnosis in FASD

Despite the importance of early diagnosis in FASD, many challenges remain. First, confirmation of the precise amount of prenatal alcohol exposure is typically based on maternal self-reports and questionnaires and this fails to precisely define the exact amount of prenatal alcohol exposure. For example, the IOM provides a vague definition of significant exposure to alcohol, considering this “a pattern of excessive intake characterized by substantial, regular intake or heavy episodic drinking”. Also, the mother may not accurately present information of alcohol consumption due to memory recall problems, as well as denial, lack of awareness, or fear of losing her child (Welch-Care, 2005). Furthermore, in many cases, information about prenatal alcohol exposure is obtained from third-party sources such as social workers who may report only the visibly severe cases of FAS and pFAS but not ARND. Current research on identifying the by-products of alcohol metabolism that remain in the body tissues for weeks (Pragst and Yegles, 2008) may help to circumvent the problem of inaccurate reporting of alcohol use during pregnancy. Nevertheless, defining the precise timing and amount of prenatal alcohol exposure remains a significant issue in human studies of FASD.

A further problem in FASD diagnosis is that it is also difficult to detect individuals with ARND because they do not show the physical abnormalities that warrant a diagnosis. Moreover, because the facial abnormalities become less distinctive or differ with age (Streissguth et al., 1991), many individuals with ARND are misdiagnosed or undiagnosed, even when they present with behavioural problems later in adolescence. Without a confirmed
history of prenatal alcohol exposure, these individuals cannot be given a diagnosis and access to the crucial interventional services they need.

Another challenge lies in differentiating non-dysmorphic patients such as those with ARND from individuals with other developmental disorders that share common diagnostic features. For example, attention problems, low IQ, and facial abnormalities are also found in patients with developmental disorders such as William’s Syndrome or high and low functioning autism. In fact, without a history of prenatal alcohol exposure, many individuals with FASD are initially misdiagnosed with attention-deficit/hyperactivity disorder (ADHD). Accordingly, they are unable to access early intervention services for FASD and crucial time for rehabilitation is lost (Nash et al., 2006) Thus, it is essential to establish a disorder-specific brain and behavioural profile that can be used to detect FASD, particularly in those patients lacking physical abnormalities.

Finally, physicians’ lack of knowledge, skill, and training in FASD and the varying diagnostic systems between countries and medical centers has created discrepancies in the diagnosis of FASD. Inaccuracies in diagnosis decrease the power needed to make clinically meaningful comparisons between subgroups within the FASD spectrum (Astley and Clarren, 2000). This calls for a universal diagnostic approach to bring about greater consistency clinically and in research.

In view of the great importance in detecting ARND at an early stage combined with diagnostic inconsistencies and challenges, further research into the unique features of this FASD subgroup is warranted.
2.2 Alcohol’s Teratogenicity on the Developing Brain

2.2.1 Mechanisms of Action

Effects on Neuronal Proliferation

Alcohol readily enters the maternal bloodstream, crosses the placenta, and exerts direct effects on the developing embryo or fetus. Animal models and tissue cultures have been particularly useful for a controlled study of the mechanisms underlying alcohol’s adverse effects. These studies have shown that alcohol causes damage to the brain through neuronal cell death and disrupted neuronal migration.

First, alcohol causes cell death through necrosis or apoptosis. Necrosis involves the direct swelling and rupture of cells, which discharge cellular waste, causing inflammation and the death of adjacent cells. By contrast, apoptosis occurs through a slower gene-mediated process in which the damaged neuronal cells shrink and the internal DNA breaks up into fragments. The cells then split into multiple apoptotic bodies, which are subsequently engulfed by scavenger cells.

Ethanol-induced apoptosis and necrosis can occur through several processes. One of the most common mechanisms is through alcohol’s production of free radicals. Free radicals are highly reactive oxygen species that create oxidative stress (Bredesen, 1996). Antioxidants provide a protective effect by eliminating these free radicals (Heaton et al., 2000). However, alcohol not only leads to an increase in free radicals, but also reduces the number of antioxidants, further increasing the level of oxidative stress. Ultimately, this damages the cell’s internal components such as proteins and DNA. Among the most critical cell organelles that are affected is the mitochondria, which regulates the cell’s energy. In the
mitochondria, alcohol-induced oxidative stress disrupts internal calcium levels, ultimately leading to necrosis and apoptosis (Kroemer et al., 1997).

In addition, alcohol exposure can cause cell death by inducing excitotoxicity, or the increased activity of neurotransmitters, which leads to excessive activation of receptors (Michaelis and Michaelis, 1994). In particular, prenatal alcohol exposure has been shown to disrupt the glutamate and NMDA receptor system, which are critical for establishing neuroplastic changes during development (McDonald and Johnson, 1990). Alcohol serves as a competitive inhibitor of glutamate and directly binds to the NMDA receptor (Riley et al., 2001). This process causes an increased sensitivity and up-regulation of NMDA receptors. When the alcohol is removed, there is an increased activation of NMDA by glutamate, causing a surge of calcium influx and cell death via necrosis or apoptosis (Choi, 1995; Pang and Geddes, 1997)

Furthermore, alcohol interferes with the production of cells by altering the cell cycle. Preliminary evidence suggests that ethanol prolongs the gap phase of the cell cycle particularly G1 and inhibits the progression of cells into the mitotic phase (Mikami et al., 1997).

In terms of timing, animal studies have established that alcohol exposure in the first few weeks of embryogenesis can cause apoptosis of cranial neural crest cells (Cartwright et al., 1998) that ultimately results in facial abnormalities. At this early stage, alcohol also kills stem cells that are precursors to cortical neurons (Miller, 1989). Subsequent to this phase, alcohol may additionally selectively kill cells in different regions of the brain such as the hypothalamus, corpus callosum, and regions of the frontal lobe (De et al., 1994; Guerri et al., 2009).
**Effects on Neuronal Migration**

The second major process that is affected by alcohol exposure is neuronal migration. Ethanol interferes with radial glial cells, cell adhesion molecules, cytoskeletal proteins, and growth factors, all of which are crucial in the guidance of neuronal cells to their appropriate locations in the brain.

A major target of alcohol’s teratogenicity is the radial glial cell. These radial glial cells form tracks along which neurons move to their appropriate locations within the cerebral cortex. Thus, these cells are crucial for the formation of the cortical architecture. Once neuronal migration is complete, the radial glial cells become astrocytes, which serve to support growth and development. Interestingly, animal models suggest that alcohol causes radial glial cells to convert to astrocytes much sooner, and critically before neurons have completed their migration to the cortex (Miller and Robertson, 1993). This therefore results in displaced neurons that are stranded deep within the cortical layers as well as superficially.

In addition, alcohol disrupts the cell adhesion proteins that help bind migrating neurons to their radial glial guides. For instance, even small amounts of alcohol can physically block L1 cell adhesion system that normally permits neurons to communicate with each other and orient themselves through cell-to-cell contact (Ramanathan et al., 1996). A recent human study providing evidence for this latter mechanism has shown that genetic defects involving the L1 adhesion molecule produce abnormal neurodevelopment. Importantly, the brain abnormalities observed in this condition were similar to those in individuals with FASD (Yeaney et al., 2009).

Ethanol has also been shown to alter the subunits of cortical integrin (Siegenthaler and Miller, 2004), another crucial cell adhesion protein involved in neuronal migration. Integrin
binds to the edge of migrating neuronal cells until there is a calcium influx within the cell (Lawson and Maxfield, 1995), at which point the cell is released. Calcium levels are mediated by NMDA receptors, which alcohol competitively inhibits. As such, ethanol-changes in calcium levels further prevent migration by interfering with the release of migrating neurons from integrin.

Prenatal alcohol exposure also adversely affects cytoskeletal proteins such as actin and microtubules, which are necessary for the reorganization and migration of neurons (Marin and Rubenstein, 2003). Ethanol has been shown to disrupt the integrity and organization of actin in a concentration-dependent manner (Allanson et al., 2001). The polymerization of microtubules is also altered through prenatal exposure to alcohol (Yoon et al., 1998). Finally, alcohol interferes with the neuronal growth factors (Borghesani et al., 2002) and also decreases the expression of genes that guide development (Peng et al., 2004), leading to further errors in neuronal migration. Ultimately, alcohol’s interference with neuronal migration may cause neurons to be stranded in the deep cortical layers IV, V, and VI or in some cases the neurons over-migrate into layer 1 (Miller, 1986).

**Genetic Mechanisms**

Both animal and human studies establish that alcohol interacts with the genetic susceptibilities of the mother and child to induce diverse effects (Green et al., 2007). Animal research suggests that genetic differences affect the fetus’ susceptibility to the teratogenic effects of prenatal alcohol exposure (Chernoff, 1977). Similarly, human studies show that if a mother possesses a specific alcohol metabolism gene variant (i.e. ADH1B*3), her child may be less vulnerable to the adverse effects of alcohol (Jacobson et al., 2006). Alcohol has also been found to affect the genes that mediate the development of the face and brain. For
example, alcohol interacts with genes in the Hedgehog signaling cascade such as the one that
governs sonic hedgehog molecule, resulting in a wide spectrum craniofacial abnormalities
(Chen et al., 2000).

Alcohol can also have epigenetic effects, which involves an alteration of gene expression
without a change in the underlying genetic sequence. Specifically, alcohol can induce these
effects through interference with DNA methylation (Mato and Lu, 2007), modification of
histones (Shukla et al., 2008), as well as chromatin remodeling processes (for a review, see
Ramsay, 2010).

2.2.2 Quantity, Frequency, and Timing of Alcohol Exposure
It is well established that alcohol’s damage to the developing brain varies as a function of
quantity of alcohol consumed, frequency of consumption, and timing of fetal development
(Sulik et al., 1986; May, 1995).

In terms of quantity, binge drinking (4 or more drinks per occasion) has been shown to
correlate highly with the prevalence rate of the most visibly severe cases of FASD (May et
al., 2008). The high blood alcohol levels produced by this pattern of drinking result in the
most severe cognitive and behavioural outcomes (West and Goodlett, 1990; Jacobson and
Jacobson, 1994). Moreover, large-scale population studies have shown that in countries
where there is a high prevalence of binge drinking (e.g. South Africa), there is
correspondingly a higher rate of FAS (May et al., 2007); In contrast, countries where
drinking patterns are more moderate (e.g. United States) have a greater prevalence of non-
dysmorphic individuals such as those with ARND (May et al., 2006).
The frequency of alcohol consumption also has a profound impact on the severity of fetal effects. Mothers who drink regularly over the course of pregnancy (i.e. 2 days/week) and not just on a few occasions are more likely to produce children with severe physical abnormalities (FAS or pFAS) (May et al., 2007).

Importantly, the timing of alcohol exposure is critical because different brain regions and craniofacial features develop at different stages during pregnancy. For instance, if blood alcohol levels are high when the craniofacial structure is forming, facial anomalies will likely result. In fact, recent evidence in rodents shows that while early exposure results in pattern of facial defects seen in FAS, exposure two gestational days later results in a completely different facial phenotype (Godin et al., 2010).

Thus, in evaluating the effects of alcohol exposure on the developing brain, it is important to consider the quantity, timing, and frequency in combination to most accurately predict outcomes.

2.2.3 Animal Models of Prenatal Alcohol Exposure

To date, widespread debate exists as to whether the brain abnormalities observed in patients with FASD reflect the teratogenic effects of prenatal alcohol exposure exclusively or other confounding factors associated with the wide range of adverse conditions to which children with FASD are typically exposed in early life (Guerrini, 2007). These adversities include the multiplicity of factors associated with poverty; prenatal exposure to cigarette smoking and drugs as well as later second-hand exposure effects; malnutrition; and stress. Notably, all of these factors can cause cell damage in the developing brain (Abel and Hannigan, 1995). Importantly, the animal studies, which have allowed for the control of many of these
confounding variables, have established that alcohol is definitely the major contributor to the deleterious effects of prenatal alcohol exposure on the brain.

Early studies using animal models such as rats, mice, and dogs showed a clear link between prenatal alcohol exposure and the emergence of the physical and neurobehavioural abnormalities seen in humans (Chernoff, 1977; Abel and Dintcheff, 1978; Ellis and Pick, 1980). One study in fact showed humans and rodents exposed to alcohol in utero had similar deficits in learning, attention, executive functioning, and motor skills (Driscoll et al., 1990).

More recently, rodent studies using magnetic resonance microscopy (MRM) have helped to further delineate the structural defects in the developing brain of prenatally exposed animals (Parnell et al., 2009; Godin et al., 2010; O’Leary-Moore et al., 2010). These studies have produced high-resolution images of the animal models of FASD through the use of high-field strength magnets (above 7 T), custom coils designed for small animals, and contrast agents that enhance the contrast of structures (Petiet et al., 2007). In these studies, pregnant mice were injected with high doses of alcohol (blood alcohol concentration of 350-440 mg/dl) on gestational days 7, 8, and 10 (G7, G8, G10), which approximate days 17, 21, and 29 of human pregnancy. When examined on gestational G17, these mice exhibited a broad spectrum of facial and brain defects.

Notably, the most severely affected were mice exposed to ethanol on G7, which is equivalent to day 17 of human pregnancy (Godin et al., 2010). These animals showed holoprosencephaly (HPE), which is characterized by facial abnormalities (including a narrow face), forebrain reductions, corpus callosum deficiencies, and an improper union of the two hemispheres. They also showed significantly reduced cerebral volumes, absent olfactory
bulbs, and decreased frontothalamic and overall brain width, as well as increased size of lateral ventricles.

In mildly affected cases with little facial dysmorphology (equivalent to ARND in children), cerebral volume reductions were less than the dysmorphic mice (Godin et al., 2010). Specifically, olfactory bulbs were present but reduced in size and the space between the cerebral hemispheres still remained wide relative to controls. There was also evidence of an enlarged third ventricle. Interestingly, the non-dysmorphic alcohol-exposed mice additionally showed evidence of displaced cells or cortical heterotopias, likely due to alcohol’s interference with the radial glial cells that mediate cell migration (Godin et al., 2010). These heterotopias have been previously shown to be associated with the emergence of seizures, which are sometimes present in individuals with FASD (Sun et al., 2009).

Like G7 mice, the G8 mice (equivalent of day 21 of human pregnancy) showed volume reductions in the olfactory bulbs, hippocampus and cerebellum, particularly in the right hemisphere. They also showed an enlargement of the third ventricles, corresponding with a reduced transverse cerebellar diameter (Parnell et al., 2009). However, the G8 mice showed a completely different facial phenotype than the G7 mice and the G8 face was consistent with DiGeorge’s syndrome.

In addition, ethanol exposure later in gestation at around G10 (which is equivalent to day 29 of human pregnancy) led to a further reduction of the cortical grey matter volumes beyond total brain volumes and the formation of heterotopias (O’Leary-Moore et al., 2010). There was also evidence of an enlarged third ventricle. However, by this stage, mice did not show any significant facial abnormalities.
The combination of these findings therefore suggests that within the first four weeks of pregnancy, prenatal alcohol exposure can induce a spectrum of abnormalities, including significant brain damage in the absence of any major physical facial dysmorphology. This is particularly significant because it is during this time frame that many women, unbeknownst to their unplanned pregnancy, drink heavily.

2.2.4 Human Neuroimaging Studies of FASD

*Structural MRI*

In humans, the advent of magnetic resonance imaging (MRI) has allowed for *in vivo* examination of the effects of prenatal alcohol exposure on brain structure and function. In principle, MRI captures the properties of brain tissue by measuring the dynamics of hydrogen atoms (protons) in water found abundantly in the brain.

When an individual is inside an MRI scanner, the magnetic moment of hydrogen atoms in the brain’s water content align with the field of the scanner. Subsequently, when a radio wave is applied, the hydrogen atoms spin to the opposite direction. When the radio frequency is turned off, the atoms rotate back to their normal position, emitting energy. This generates an MR signal based on the spin relaxation, which is the time it takes for the atoms to relax from their rotated position to their original position. This spin relaxation is described using relaxation constants including $T_1$, $T_2$, which reflect the properties of the tissue and are used to classify the various tissue types (i.e. grey matter) to construct an image of the brain. From these images, brain volumes, surface area, and thickness of various structures can be examined using specialized computer technology. This technology has provided a lens for looking into the global and regional brain abnormalities in humans with FASD.
**Global Abnormalities**

Consistent with animal research, MRI research has shown that individuals with FASD exhibit reduced total brain volumes (Archibald et al., 2001; Astley et al., 2009; Mattson et al., 1996). Also seen in this population have been decreases in the volumes of both white and grey matter within the cortex (Archibald et al., 2001; Sowell et al., 2001a; Mattson et al., 1994; Bjorkquist et al., 2010). However in most studies, reduced cortical volumes did not remain significant once total brain volumes were controlled for, indicating a global effect of prenatal alcohol exposure on the developing brain.

**Corpus Callosum**

In addition to the above global effects, previous studies have shown regional abnormalities in the brains of individuals exposed to alcohol in utero, particularly in midline regions. In keeping with the animal findings, the corpus callosum has been found to be a particularly vulnerable region that is often absent (agenesis) or decreased in size among individuals with prenatal alcohol exposure (Astley et al., 2009; Riley et al., 1995). The largest effect is in the posterior splenium region of the corpus callosum (Autti-Ramo et al., 2002; Riley et al., 1995). Also noted are alterations in shape (Bookstein et al., 2002b) and position of this structure (Sowell et al., 2001a). For example, Sowell et al. (2001a) reported that the more anteriorly displaced the person’s corpus collosus, the greater the impact on verbal learning. Such corpus callosum aberrations have also been shown to be linked to problems with executive functioning (Bookstein et al., 2002b) and motor coordination in FASD (Roebuck-Spencer and Mattson, 2004).
Subcortical Structures

Many subcortical structures also show increased susceptibility to prenatal alcohol exposure. Studies indicate that in FASD, the size of the basal ganglia are reduced, especially the caudate, which shows decreases even when total brain volume is considered (Archibald et al., 2001; Astley et al., 2009; Mattson et al., 1992; Nardelli et al., 2011). Caudate abnormalities have been linked to deficits in executive functioning, attention, and response inhibition (Mattson et al., 1996). There is also evidence of reduced volumes of other basal ganglia structures such as the globus pallidus (Nardelli et al., 2011; Roussotte et al., 2011), putamen (Astley et al., 2009; Nardelli et al., 2011; Riikonen et al., 2005; Roussotte et al., 2011), and lentricular nucleus (globus pallidus and putamen) (Mattson et al., 1996).

Individuals with prenatal alcohol exposure also present with structural abnormalities of the diencephalon, although results are inconsistent for these structures. For example, the diencephalon (composed of thalamus and hypothalamus) was significantly reduced in some studies (Mattson et al., 1996) but not others (i.e. Archibald et al., 2001). In line with these inconsistencies, a component of the diencephalon, the thalamus, was shown to be either unaffected after controlling for total brain volume (Archibald et al., 2001; Roussotte et al., 2011) or significantly reduced beyond the global reductions in volume (Mattson et al., 1992; Nardelli et al., 2011).

Cerebellum

As reported above, the cerebellum is one of the structures most significantly affected in fetal alcohol affected individuals. The findings from this research have revealed reductions in both volume (Mattson et al., 1994; Archibald et al., 2001; Astley et al., 2009; Bookstein et al., 2006) and surface area (Autti-Ramo et al., 2002). The cerebellar reductions were shown to
reflect a smaller anterior vermis in individuals exposed to alcohol prenatally (O’Hare et al., 2005; Sowell et al., 1996), consistent with early animal studies showing Purkinje cell loss in this region (Goodlett et al., 1990). Also noted was a significant displacement of the anterior vermis in the inferior and posterior direction. Regarding the cognitive implications of these abnormalities, a negative correlation was observed between the degree of displacement of the superior edge of the anterior vermis and verbal learning ability in individuals with FASD, such that the greater the displacement of the structure was associated with poorer performance in this domain (O’Hare et al., 2005).

**Cerebral Cortex**

**Frontal Lobe**

Frontal lobe abnormalities, which reflect decreased volumes as well as reduced grey matter (Astley et al., 2009; Sowell et al., 2002a), are commonly reported among individuals exposed prenatally to alcohol. In one study, frontal lobe volume reductions represented a global effect since they were spared once total brain volume was controlled (Archibald et al, 2001). However, other studies have found local reductions in frontal lobe volumes, particularly in the left ventral frontal regions (Sowell et al., 2002a). Moreover, frontal lobe volumes have been shown to be a good indicator of disease severity with one study showing that reductions were significantly larger in individuals with FAS as compared to more mildly affected subgroups within the fetal alcohol spectrum (Astley et al., 2009).

In addition to these imaging findings, case reports have indicated abnormal anatomy of the frontal lobes on autopsy. Observed abnormalities included atrophy (Riikonen e al., 2010) and microgyria on the superior frontal gyrus (Reinhardt et al., 2010).
Temporal Lobe

Consistent with the findings of frontal lobe atrophy, temporal lobe volumes are also significantly reduced in alcohol-exposed individuals; however this effect may not seem to persist once total brain volumes are controlled for (Archibald et al., 2001). Other brain pathology studies of the temporal lobes have shown decreases in grey matter of the temporal lobes beyond total brain volume (Sowell et al., 2002a; Li et al., 2008). For example, local reductions were found in the fusiform gyrus of the temporal lobe (Coles et al., 2011). In addition, evidence also exists showing narrow (Sowell et al., 2002a) and displaced temporal regions (Sowell et al., 2002b).

The limbic areas of the temporal lobe are also greatly susceptible to damage due to prenatal alcohol exposure. In both adolescents and adults with FASD, the hippocampus has been shown to be relatively smaller than normal, even after correcting for total brain volume (Willoughby et al., 2008; Nardelli et al., 2011). In addition, the amygdala, which is an important limbic structure for social and emotional processing (Olsen, 2007), has been found to be altered in one study (Nardelli et al., 2011), but normal in others (Archibald et al., 2001; Riikonen et al., 2005).

Parietal Lobe

The parietal lobes may be particularly vulnerable to the teratogenic effects of alcohol exposure as indicated by abnormalities of grey- and white-matter reductions, beyond total brain volume reductions. These effects are particularly evident in individuals with the FAS subtype (Archibald et al., 2001) and reflects the findings of narrow lobar boundaries and displacement of parietal regions (Sowell et al., 2002 a,b).
Occipital Lobe

Compared with the other lobar regions, the occipital lobes appear to be the least significantly affected in alcohol exposed-individuals (Archibald et al., 2011). This relative sparing of the occipital lobes may be due to the timing of occipital lobe development in relation to the time of exposure (Lebel et al. 2011). Nonetheless, a study by Li et al. (2008) showed that the occipital-temporal volumes were structurally and functionally abnormal in adolescents with prenatal alcohol exposure as compared to controls, leading to deficits in visual attention.

2.3 Normative Cortical Development

2.3.1 Determinants of Cortical Volume

Although significantly reduced cortical volumes are widely recognized in individuals with FASD (Lebel et al., 2011), the underlying determinants of these reductions have not been fully elucidated. Because cortical volume is determined by two factors, namely cortical thickness or the distance between the brain’s inner white matter surface and outer grey matter-CSF boundary (pial surface) and surface area or the 2-dimensional area, which includes both the area within the sulci and also the exposed area of the cortex known as the convex hull area, both factors need to be independently considered when discussing cortical volume. Also since gyrification or the degree of cortical folding is intrinsically linked to surface area, this index also needs to be measured when assessing cortical morphology. Gyrification is typically measured using the gyrification index (GI), which is the ratio between total surface area and the convex hull area (Van Essen and Drury, 1997) and in a two-dimensional analysis, GI translates to the ratio between the inner and outer brain contours (Zilles et al., 1988). The relationship between the various cortical determinants is illustrated in Figure 1.
Figure 1 Determinants of cortical volume and related factors
2.3.2 The Development of Surface Area and Cortical Thickness

Cellular Mechanisms

Not only do cortical thickness and surface area refer to different structural aspects of the cortical volume, they also reflect different biological mechanisms. During the course of human evolution, expansion of the cerebral cortex was driven more by an increase in surface area than cortical thickness (Rakic, 1995). Previous studies have also shown that variation in cortical volumes between individuals represents differences in surface area as opposed to cortical thickness (Pakkenberg and Gundersen, 1997).

Cortical thickness and surface area are also mediated via different cellular processes. Past studies have helped elucidate the processes of corticogenesis through various methods including counting of mitotic figures, immunohistochemical identification of cells, and autoradiographic studies using \([^{3}H]\) thymidine DNA replication markings (Chan et al., 2002). These studies have established that surface area develops within the first 6 weeks of human embryogenesis when progenitor cells divide in the ventricular zone. During this time period, progenitor cells divide symmetrically to produce identical daughter cells, which then become the founders of the ontogenetic columns that define the magnitude of cortical area (Rakic, 1974). The cells then align vertically along the ventricular surface in proliferative radial units, which consist of up to 5 progenitor cells in early stages, and as many as 12 later in development (Rakic, 1995). The number of radial units indicates the number of projections to the cortical plate and, consequently, the surface area of the brain (Rakic, 1974).

Cortical thickness begins to develop at the end of the first trimester around the 12th week of development when a peak in neuronal proliferation within the cortex is seen (Simonati et al., 1999). This surge emerges from a switch to asymmetric division of the progenitor cells, each
of which produces one identical progenitor cell and one post-mitotic neuron. The progenitor daughter cells adhere to the cortical surface and undergo further mitotic division, whereas the post-mitotic neurons migrate to the cortical plate through shafts of radial glial cells (Rakic, 1995). However when neuronal migration is complete, the glial cells become astrocytes (Miller and Robertson, 1993). As mentioned previously, alcohol can cause these radial glial cells to convert prematurely into astrocytes, thus disrupting the migration of post-mitotic neurons to the cortical plate and leading to the heterotopias (displaced cells) observed in mouse models of FASD.

After migration through the radial shafts, the post-mitotic neurons settle at the cortical plate in an “inside-out” gradient in which early neurons form deep layers with the later-generated neurons settling in more superficial regions (Rakic, 1988). According to the radial unit hypothesis (Rakic, 1988), the progenitor cells within a radial unit of the ventricular zone reach the cortical plate successively and eventually arrange into the same ontogenetic column of the cortex (Mountcastle, 1979). Within each ontogenetic column, the density of cells (e.g. neurons and glial cells) contributes to the thickness of the cortical grey matter with this value varying among different brain regions. In the monkey embryo, for instance, the primary visual cortex has a much denser distribution of cells than other regions reflecting the extended period of neurogenesis over the course of two months in the primary visual cortex (Rakic, 1974). Vasculature and dendritic and synaptic processes that come from intrinsic connections between neurons in the cortical column further contribute to the thickness (Gogtay et al., 2004).

Importantly, while prenatal development contributes greatly to the establishment of the ontogenetic columns, cortical thinning occurs postnatally due to dendritic arborization and
pruning processes (Huttenlocher, 1990) as unused neuronal connections are eliminated. Alternatively, it has been suggested that over development, cortical grey matter decreases may be observed as a result of increased myelination of cortical fibres (Paus et al., 2008). In other words, as the myelination of the intra-cortical fibres increases, the grey matter density appears to be less, manifesting on MRI scans as a thinner cortex.

Thus, as shown in Figure 2, surface area is defined by the number of proliferative radial units established at the beginning of embryogenesis, whereas cortical thickness reflects the density of cells within each proliferative unit. Because surface area and cortical thickness are each determinants of cortical volume and reflect different evolutionary and cellular processes, independent investigation of these parameters is needed to fully understand the effects of prenatal alcohol exposure on the cortex.
**Figure 2** Corticogenesis processes underlying the formation of cortical area and thickness

A) Surface area is formed in the first 6 weeks of development when progenitor cells in the ventricular zone undergo symmetric division and form radial units (*red columns*) that are precursors to cortical neurons. The number of radial units establishes the magnitude of cortical area. B) Cortical thickness is formed when progenitor cells undergo asymmetric division and produce post-mitotic neurons that migrate to the cortical plate via radial glial cells to build the ontogenetic columns (*orange columns*) that form the layers of the cortex and partly establish the thickness.
**Genetic Underpinnings**

The different cellular processes of cortical thickness and surface area reflect different genetic mechanisms. Indeed, Panizoni et al. (2009) showed a lack of correlation between cortical thickness and surface area, indicating independent governing genetic mechanisms.

Accumulating evidence also suggests that some genes affect cortical surface area with little effect on thickness, while others are primarily implicated in thickness and not surface area.

A host of candidate genes including *MCPH1* to *MCPH7* (Rimol et al., 2010) on chromosome 8, X-linked *MECP2* (Joyner et al., 2009), and *KCTD8* on chromosome 4 (Paus et al., 2011) have been found to affect cortical surface area and total brain volume more so than thickness through various mechanisms.

The genes implicated in primary microcephaly, namely Microcephalin (*MCPH1*), *CDK5RP* (*MCPH3*), *ASPM* (*MCPH5*) and *CENPJ* (*MCPH6*) are involved in regulating aspects of cell division and cell cycle division (Mochida, 2009). Moreover, animal models show that these genes are expressed in the neuroepithelium of the ventricular zone during cortical development (Woods et al., 2005). As such, they are potentially involved in regulating the symmetrical division of progenitor cells that form the precursory radial units constituting surface area. This explains why loss of function in these candidate genes leads to a dramatic reduction in surface area and total brain volume (microcephaly) with little effect on cortical thickness. Furthermore, human studies show that these genes differentially affect males and females in terms of these cortical area reductions (Rimol et al., 2010) with *MCPH1* affecting females primarily and *CDK5RP* affecting males.

In addition, the X-linked *MECP2* gene is involved in cell division and affects cortical surface area and total brain volume in a similar manner to the microencephaly genes (Joyner et al.,
However, this gene was found to affect cortical surface area only males, possibly due to buffering effect of the extra X-chromosome in females.

More recently, human studies showed that the $KCTD8$ gene is linked to surface area and cortical folding but not cortical thickness in females, albeit through a different mechanism than the microcephaly genes and $MECP2$ (Paus et al., 2011). Specifically, $KCTD8$ is involved in the formation of potassium channels that may mediate apoptosis of neuronal cells. Thus, a loss-of-function in this candidate gene could result in abnormal apoptosis of progenitor cells in the initial phase of corticogenesis, significantly altering the surface area of the cortex.

In contrast, mutations in other genes such as $PAX2$ and $TBR2$ have been shown to significantly alter cortical thickness without much affect on surface area (Glaser et al., 2004; Baala et al., 2007). These genes are also involved in processes neuronal migration and neuronal fate determination, and therefore are potentially implicated in the migration of post-mitotic neurons to the cortical plate, which results in the formation of the ontogenetic columns of the cortex.

Thus, the dissociation between the development of cortical surface area and thickness is in part governed by independent genetic mechanisms. Notably, many of these global genes affect females and males differentially, further contributing to variation in cortical morphology.
2.3.3 The Development of Gyrification

Cellular Mechanisms

In the third trimester of human development, the brain undergoes an exponential increase in volume (Chi et al., 1977) that necessitates folding of the cortical surface or gyrification (Armstrong et al., 1995). It was formally believed that gyrification is the result of mechanical friction between an expanding brain and the confines of the skull (Le Gross Clark, 1945). However, later explanations suggest that gyrification is a result of internal cortical mechanisms as opposed to external constraints. One current theory suggests that during the period of rapid brain development in the third trimester, the outer cortical layers grow more quickly than the inner layers, and this results in a buckling of the cortical surface (Van Essen and Drury, 1997; Toro and Burnod, 2005). Another theory proposes that neuronal connections forming during the second trimester of development create tensions between cortical fibers, resulting in the formation of cortical folds that manifest on the surface (Van Essen and Drury, 1997). Regions close together form gyri, while more wildly separated regions become sulci. As a consequence, cortical folding allows for close connectivity of related regions of the brain, leading to greater efficiency in signaling pathways.

A loss of gyrification or lissencephaly has been previously shown to be associated with errors in neuronal migration (Kato and Dobyns, 2003), although the full mechanism is unknown. As such, teratogens such as alcohol can adversely affects the gyrification process by interfering with radial glial cells, cell adhesion molecules, microtubule elements, and growth factors that mediate cell migration.
Genetic Underpinnings

Alcohol may also interact with the fundamental genes that govern the processes underlying gyrification. Indeed, past research has shown that disruptions of gyrification in lissencephaly are caused by sporadic mutations in the LIS1 gene (Lo Nigro et al., 1997) as well as the X-linked DCX doublecortin gene (Gleeson et al., 1998).

Both LIS1 and DCX have been shown to mediate microtubule processes that mediate neuronal migration from the ventricular zone to the cortical plate. Specifically, LIS1 governs the microtubule motor protein, dynein, which guides the neuronal cells to their appropriate locations in the cortex (Faulkner et al., 2000). Doublecortin (DCX) is involved in microtubule stabilization and binding, again affecting the migration of cells destined for the cortex (Gleeson et al., 1999).

2.3.4 Timing of Corticogenesis

Monkey studies have helped to understand the developmental timing of the various cortical features – surface area, cortical thickness, and gyrification. Algan and Rakic (1997) used ionizing radiation to selectively kill cells that were in their preliminary stages of development while sparing more mature post-mitotic neurons. These researchers showed that exposure to radiation early in embryogenesis before E40 (corresponding to 6 weeks into human fetal development) resulted in a decrease in number of founder cells in the ventricular zone, thereby affecting the cortical surface area more than cortical thickness and the cytoarchitectonic features of the cortex. This may be because early death of precursor cells reduces of the number of radial units forming surface area and by consequence the number of oonogenetic columns.
On the other hand, irradiation of the monkey embryo after E40 resulted in the death of post-mitotic neurons destined for the cortical plate, thus resulting in a decrease in cortical thickness and the alterations of cortical cell layers (Barondes et al., 1997). Additionally, the irradiation occurring during the asymmetric division phase of cortical development disrupted gyrification of the brain (Stewart et al. 1975).

The above findings show that different cortical features undergo growth at different points during prenatal development and may signify potentially different effects depending on timing of alcohol exposure. While surface area is largely established within the first 6 weeks of embryogenesis, cortical thickness can undergo significant modifications from 6 to 17 weeks into human development (Rakic, 1995). The 6 to 17 week phase is also crucial for the neuronal migration and the establishment of cortical folding. The thickness and gyrification of the cortex continue to undergo modifications postnatally, but rely on the precise execution of prenatal cortical development.

2.4 Factors Affecting Cortical Development

2.4.1 Age-Related Changes in Cortical Development

Throughout childhood and adolescence, the brain undergoes significant developmental change in a very characteristic pattern. Lenroot et al. (2007), for example, conducted a longitudinal MRI study on a large sample of 387 human participants aged 3-27 years. Findings revealed that total brain volume followed a curvilinear trajectory, which peaked at the time of puberty. Correspondingly, an earlier study by this same group found that cortical grey matter volume developed in a curvilinear trajectory between 4 to 22 years of age, reflecting a preadolescent increase and postadolescent decrease (Giedd, 1999).
On a regional level, different areas of the brain undergo change over slightly varied developmental time courses. The maturation of the grey matter first occurs in areas involved in basic processes, including motor and sensory brain areas, frontal areas recruited in taste and smell functions, the primary visual cortex of the occipital lobe (Gogtay et al., 2004). Regions involved in more complex processing such as executive functioning develop later, following a parietal to frontal sequence of maturation. The last region to develop is the heteromodal association areas of the temporal lobe (including the posterior aspect of the superior temporal sulcus, superior temporal gyrus, and middle temporal gyrus), which are involved in the integration of object-recognition, memory, and other functions. Brain maturation also develops from evolutionarily older regions to more recently evolved regions such as the inferior temporal cortex, superior temporal gyrus, posterior parietal cortex, and prefrontal cortex (Gogtay et al., 2004).

Consistent with changes in cortical grey matter, the thickness of the cortex also shows an increase peaking at puberty followed by a decrease in adolescence that becomes stabilized in adulthood (Shaw et al., 2008). The Shaw et al. (2008) study revealed two principles of cortical thickness development: First, age-related trajectories are regionally specific showing either linear, quadratic or cubic trends and different peaks for different brain areas. Specifically, lateral frontal, lateral temporal, parietal and occipital areas follow an S-like cubic pattern of development, whereas the insula and anterior cingulate develop in a quadratic manner and the orbitofrontal region exhibits a linear trend. Second, peak cortical thickness development varies significantly by region with the somato-sensory and occipital regions developing first (~7 years) and hetermodal areas such as the parietal-occipital regions developing later (~9-10 years) while higher-order cortical areas such as the dorsal lateral prefrontal cortex form last (~10.5 years).
In cases of brain dysfunction the peak of the cortical thickness trajectory may be altered. For example, in attention deficit hyperactivity disorder (ADHD), which is often comorbid with FASD, the median age at which half the cortical points reached their peak thickness was 10.5 years for the ADHD group and 7.5 years for typically developing controls, suggesting a delay of approximately three years (Shaw et al., 2007). Interestingly, Shaw et al. (2007) also showed that the delay in cortical thickness development was found mostly in the lateral prefrontal cortex, particularly in the superior and dorsolateral prefrontal cortices. The temporal regions also showed a significant delay in cortical development, particularly in the posterior portions of the middle and superior temporal gyrus, which are higher-order heteromodal association areas.

Possible Underpinning of Age-Related Grey Matter Changes

The increase and subsequent decrease of cortical grey matter may reflect different molecular events, although the precise mechanism is not fully understood. The initial phase of cortical thickening from birth well into childhood is hypothesized to represent re-modeling of the prenatal ontogenetic columns. Preliminary evidence for this theory comes from experimental animal model studies that show that depriving one eye of sensory input leads to increased thickness of ocular dominance columns serving the other eye (Antonini and Stryker, 1996). Other studies also propose that an increase in dendritic and axonal connections between neurons within each column contributes to the increased cortical grey matter during early childhood development (Chklovskii et al., 2004).

On the other hand, the decrease in cortical volumes and thickness in adolescence may be the product of synaptic pruning (Huttenlocher and Dabholkar, 1997) when neurons and synapses become eliminated as the most important connections are formed. However, another theory
holds that the greater contributing factor to the decrease in cortical grey matter density observed on neuroimaging may be related to an increase in intra-cortical myelination (Sowell et al., 2004). White matter has been found to increase during adolescence (Lenroot et al., 2007) possibly due to increased axon caliber (Perrin et al., 2008). This increase white matter volume at the white matter-grey matter interface could manifest as a reduction in cortical grey matter on MRI scans (Paus et al., 2008).

2.4.2 Age Trajectories in FASD

Age-related trajectories of cortical development have generally not been investigated in FASD. One exception is a human morphology study by Zhou et al. (2011), which showed that while individuals with FASD underwent cortical thinning with age similar to controls, groups differed in regions of cortical thinning. Within the FASD group, cortical thinning was observed in the bilateral precentral gyrus and inferior temporal lobe (including the fusiform gyrus), whereas in typically developing individuals, cortical thinning was in the bilateral frontal and occipital gyri. However as this study was cross-sectional in nature and included a mixed sample of only 33 individuals, there was not sufficient power to detect true and generalizable age-related trajectories in this clinical population.

2.4.3 Sex Dimorphism in Normative Development

Sex hormones are known to profoundly affect the development of brain structure. These hormones act on sex receptors, which are particularly abundant in the cortical regions (Finley and Kritzer, 1999). Sex differences can arise both during the prenatal period when fetal androgens induce permanent effects on the brain, and postnatally, especially during puberty (Paus, 2010). On a molecular level, sex hormones alter neuronal survival, neurogenesis,
neurite outgrowth, and synaptogenesis (for a review, see Romeo and McEwen, 2004), and ultimately have a major impact on brain structure.

A review of MRI data investigating sex differences in brain structure (Paus, 2010) showed that across development, total brain volume in males was consistently higher than in females, even when accounting for body weight. This difference ranged from 7.8% of total brain size at birth, to 11% in pre- and post-adolescence, reaching a peak of 14% at the premenopausal stage.

Consistent with the above findings on total brain volumes, cortical volumes are also smaller in females as compared to males (Lenroot et al., 2007). Further, a longitudinal study of typically developing children showed an inverted U-shape trajectory in both sexes with females peaking at 11.5 years and males at 14.5 years (Geidd et al., 1999).

More recently, Raznahan et al. (2011) showed that sexual dimorphisms in cortical volumes are driven largely by differences in the cortical determinant of surface area, particularly the convex hull area (exposed surface area) and not gyrification or cortical thickness. Specifically, Raznahan et al (2011) found that sex differences in cortical volumes emerged as a function of the delayed peak and slow decline of the convex hull area over development.

2.4.4 Sex Dimorphism in FASD

A paucity of research exists on sexual dimorphisms in FASD. Animal studies show sexual dimorphisms in stress responsiveness, hormonal activities, and behavioural indices with conflicting findings of females being affected more in some cases and males being more affected in other studies (Weinberg et al., 2008). Females exposed to alcohol prenatally generally show greater changes following an acute or short duration stressor (Weinberg,
1988), while males are more vulnerable to longer duration stressors (Weinberg, 1992).

Furthermore, a recent study showed that males exposed to alcohol in utero show greater neurogenesis in adulthood as compared to alcohol-exposed females (Coleman et al., 2012).

Limited human research exists on sex dimorphism in FASD. One study showed sexual dimorphisms in the corpus callosum with males being affected to a greater degree (Zimmerberg and Mickus, 1990). Another study found a sex by group interaction with FASD adult males showing greater reductions in cortical volumes than FASD adult females. In particular, males showed smaller grey matter volumes of the supramarginal gyrus, middle temporal gyrus, inferior temporal gyrus, transverse temporal gyrus, and lateral occipital cortex in the left hemisphere, and the supramarginal gyrus, middle temporal gyrus, caudal anterior cingulate cortex, and posterior cingulate cortex in the right hemisphere (Chen et al., 2011). Interestingly, results showed that these sex dimorphisms were observed even when controlling for birth weight, birth head circumference, adult weight, and adult head circumference (Chen et al., 2011), suggesting a specific effect on the brain independent of somatic effects.

2.5 Cortical Morphology Techniques

2.5.1 Cortical and Subcortical Analysis

In order to better understand cortical development, a number of automated computational processes have been developed to construct and analyze the cortical surface, particularly in terms of its thickness and area. One of the most widely used programs is Freesurfer (Fischl and Dale, 2000); however for the purposes of this thesis, I will provide a basic review the processes of the other commonly used pipeline, namely Corticometric Iterative Vertex-based Estimation of Thickness (CIVET) (version 1.1.10; Montreal Neurological Institute at McGill
University, Montreal, Quebec, Canada). Freesurfer and CIVET approaches are generally comparable; however, they differ in terms of their sensitivity to the extent and distribution of cortical atrophy (Lerch et al., 2005; Dickerson et al., 2009). CIVET also allows for faster processing of data than Freesurfer and has been validated over multiple databases (Lerch et al., 2005).

The CIVET pipeline includes many processes (see Figure 3): (1) preprocessing when the native MRI scan undergoes non-uniformity correction (2) registration to a stereotaxic space (3) tissue classification, (4) construction of cortical surfaces, and finally (5) computation of cortical thickness and surface area. The final stage involves smoothing of the data and non-linear surface-based registration.
Figure 3 Different stages of the CIVET pipeline
**MRI Pre-processing**

Typically, T1-weighted MRI scans are used for cortical morphology analysis because they have high spatial resolution (≤ 1 x 1 x 1 mm). Also, higher scanner field strengths (i.e. 3 Tesla > 1.5 Tesla) are preferred because they yield a greater signal-to-noise ratio, and better contrasts between white matter, grey matter, and cerebrospinal fluid. However, the increased field strength can create artifacts such as intensity non-uniformity and as geometric distortion.

Intensity non-uniformity is defined as variations in signal intensity across the scans, which arises as a result of inhomogeneities in radio-frequency signals, coil reception sensitivity inconsistencies, and undesirable interactions between the magnet and properties of the object being scanned (Vovk et al., 2007). In the initial stages of CIVET, the intensity non-uniformity is usually corrected using non-parametric non-uniform intensity normalization (Sled et al., 1998), which takes the difference of an estimation of the true scan intensities and original scan intensities in its correction. This allows for accurate classification of the various tissue types.

In subsequent steps, the non-cerebral components (i.e. the skull) are stripped (Smith, 2002), and the image is linearly registered to the ICBM 152 template (Collins et al., 1994). The process of aligning the images to a standard template allows for accurate comparisons of data from the same brain region across multiple participants.

**Tissue Classification**

After registration to the average template volume, the brain tissue is classified into grey matter, white matter, cerebrospinal fluid, and background (Zijdenbos et al., 2002; Tokha et al., 2004) using probability maps obtained from 305 pre-classified normal samples, which
indicate regions having 90% chance of belonging to a given tissue type (Zijdenbos et al., 2002). In voxels involving mixed tissue types such as deep within the sulci of the brain, a mixed model is used (Choi et al., 1991) to reflect the partial volumes.

**Construction of Cortical Surfaces**

From the classified tissue (grey matter, white matter, CSF), a Constrained Laplacian-based automated surface-extraction (CLASP) algorithm is used to construct the inner white matter surface (MacDonald et al., 2000). This involves deforming an ellipsoid polygonal model to fit the inner white matter surface. At this stage, the white matter surface is a mesh of triangular polygons (tessellated surface), each of which is defined by three vertices (See Figure 4). The white matter surface is then expanded outward to identify the grey-matter/CSF interface (pial surface) using lapacian fluid dynamics (Kim et al., 2005). The pial surface is derived from the white matter surface and therefore contains vertices corresponding to vertex points of the original surface.

**Cortical Thickness and Surface Area Analysis**

The white matter and grey matter surfaces are each defined by approximately 81,920 triangular polygons and 40962 vertices per hemisphere. The most common measure for cortical thickness is computed as the distance (defined as $d_{link}$) from a vertex point on the inner white matter surface to the corresponding vertex point on the pial surface. The correspondence is established by expanding the white matter surface to the pial surface such that the surfaces are aligned in terms of their vertices and topology (Lerch and Evans, 2005). This method provides an estimate of the thickness of the cortex that has minimal variability.
Figure 4 Stylized rendition of cortical mesh consisting of triangular polygons defined by vertex points (marked in red)
Surface area is computed through a calculation of the 2-dimensional area of each triangle on each of the surface meshes and attributing a third of this area to the three vertices (Lyttelton et al., 2009). The cortical thickness and surface area data are then blurred. In this step, a surface-based smoothing kernel (usually between 20 and 30 mm) is used to make the data applicable to any arbitrary curved surface (Chung et al., 2004).

**Volumetric Analysis**

In addition to the cortical features, the images are warped towards segmented atlases (i.e. Collins et al., 1995) defined by complex algorithms. This allows for the identification of brain lobes and major structures and the assignment of voxels belonging to these regions. From these data, global grey-matter and white matter volumes, lobar volumes and surface area, as well as regional volumes of structures are derived.

**Non-linear surface registration**

The final phase of the CIVET pipeline involves aligning the surfaces of all the brains in the sample in order to allow for group comparisons of the data. This involves a non-linear transformation of the surfaces to a standard template. Ultimately, this serves the purpose of aligning the vertices so that group differences can be analyzed on a vertex-wise level.

### 2.5.2 Gyrification Analysis

As described previously, the traditional method of estimating gyrification, or cortical folding involved computing the gyrification index (GI), which is a measure of the ratio of the inner contour of the brain (including the exposed and sulcal contours) to the outer surface of the brain (excluding the sulci) (Zilles et al., 1988). As such, as the degree of cortical folding increases, the inner contour increases, yielding a larger value for GI.
However, the traditional method of gyrification analysis has many limitations. First, it represents a measure obtained from two-dimensional coronal sections of the brain and therefore does not account for the three-dimensional cortical surface and neglects buried sulci. Second, it provides a global measure of gyrification that may mask any regional differences in cortical folding. To account for these limitations, new 3D methods of cortical surface analysis (Fischl and Dale, 2000; Mangin et al., 2004) have been introduced to determine the ratio between the total cortical surface area and the exposed surface area on a local level. The surface ratio method (Toro et al., 2008a), another 3D local measure, was additionally developed to estimate the ratio of cortical surface area within a sphere of a given radius from each vertex point to the two-dimensional area of a circle of the same radius. The surface ratio measure of gyrification provides a simple algorithm for computation compared to other methods (Schaer et al., 2008) and allows for observation of gyrification at a local level in a 3-dimensional place.

2.6 Cortical Morphology Research in FASD

Given that cortical thickness, surface area and its associated feature, gyrification are conflated within the measure of cortical volume, it is important to examine each of these measures independently in order to fully understand the effects of prenatal alcohol exposure on the developing cortex.

Although cortical thickness has been well studied in the FASD population (with discrepant results), comparable research on surface area and gyrification is lacking. Only one study to date has shown that the brain surface extent is significantly reduced of humans especially in the orbital frontal regions (Sowell et al., 2002a). No neuroimaging studies exist on gyrification in FASD, although human autopsy studies suggest prenatal alcohol results in a
reduction of gyri and sulci in the brain as well as a disruption in cortical lamination (Clarren et al., 1978; Wisniewski et al., 1983; Konovalov et al., 1997; Roebuck et al., 1998).

However, a more comprehensive study analyzing all cortical features (thickness, surface area, and gyrification) is needed.

To date, four studies have investigated cortical thickness in FASD, but have yielded discrepant results (for a summary of studies, see Table 2). The first study by Sowell et al. (2008), which involved a sample of 21 individuals with FASD, reported cortical thickness increases of up to 1.2mm in areas of the bilateral temporal, bilateral inferior parietal, and right frontal regions including the dorsolateral prefrontal cortex. Notably, many of these areas were surrounding the perisylvian cortex (comprising of the posterior temporal and lower parietal lobes), which is involved in language processing. Children who were more mildly affected with a condition referred to as PAE ($n = 7$) (no physical dysmorphologies) also showed cortical thickening in left parietal, right ventral frontal, bilateral temporal, and dorsal frontal; however, these individuals were affected to a lesser extent than those diagnosed with FAS. This study also showed that in all alcohol-exposed individuals, the cortical thickness of the right dorsal frontal and left occipital regions was positively correlated with performance on verbal recall and visuospatial tasks. In other words, individuals with thinner cortices performed significantly worse on the behavioural measures. This finding is discrepant with the theory that cortical thinning represents increased myelination and greater efficiency and speed of processing, as suggested by Sowell et al. (2008). Ironically, the FASD group did not exhibit cortical thinning in any region examined.

Using a larger sample of 69 individuals with prenatal alcohol exposure, Yang et al. (2011) confirmed the above patterns of cortical thickening in FASD. Specifically, increased cortical
thickness was observed in superior temporal, middle temporal, and inferior frontal regions and consistent with the earlier report (Sowell et al., 2008), areas involving most significant abnormalities were those surrounding the perisylvian areas.

In line with the two aforementioned studies, Fernandez-Jaen et al. (2011) also showed that individuals with FAS who were comorbid for ADHD exhibited greater cortical thickness compared to controls and to non-alcohol exposed ADHD counterparts. These differences occurred primarily in the bilateral temporal lobes and right frontal lobe. The ADHD comorbidity in the FASD group did not significantly affect cortical thickness.

These three studies all reporting increased cortical thickness in individuals FASD (Sowell et al., 2008; Yang et al., 2011; Fernandez-Jaen et al., 2011) are consistent with early studies using voxel-based morphometry and surface-based measures to measure grey matter. In the early studies, it was found that individuals with FASD have increased grey matter density and less brain growth in the frontal and temporal regions (Sowell et al., 2001; Sowell, Thompson, Mattson, et al., 2002), with a 15% increase in the bilateral perisylvian cortex and inferior parietal regions (Sowell, Thompson, Mattson, et al., 2002).

Yet, these studies sharply contrast with a recent Canadian study by Zhou et al. (2011) showing cortical thinning in the superior and middle frontal, superior parietal, inferior temporal, and occipital regions, even after controlling for total brain volume, in FASD subjects. In particular, the thinning of temporal brain areas was opposite to the Sowell et al. (2008) findings of increased cortical thickness in the same temporal regions. Furthermore, the Zhou et al. (2011) study showed that the cortical thickness differences were similar across development. Both FASD and control groups showed an age-dependent trajectory of
cortical thinning from 6-30 years, especially in the left middle frontal gyrus, left inferior occipital gyrus, and bilateral precuneus.

Several explanations may account for the divergent findings between the first three and the fourth studies. One explanation concerns the specific diagnostic approaches used by each group of researchers. For example, Sowell et al. (2008) used the 4-Digit Diagnostic Code, Fernandez-Jaen et al. (2011) used the IOM guidelines, and Zhou et al. (2011) used the Canadian guidelines. Furthermore, the study by Yang et al. (2011) recruited a sample from three sites with different clinics, and also included a proportion of the sample that was not given a full FAS diagnosis. Second, subgroup compositions differed among studies with Sowell et al. (2008) and Yang et al. (2011) involving a larger proportion of FAS participants than Zhou et al. (2011), while the Fernandes-Jaen et al. (2011) study was comprised of individuals diagnosed with FAS exclusively. The four studies also involved different participants’ ages, which ranged from 7 or 8 to 16 years (Fernandes-Jean et al., 2011; Yang et al., 2011 respectively), 6 to 30 years (Zhou et al., 2011) and 8 to 22 years (Sowell et al., 2008), which can lead to different results because cortical thickness changes in a curvilinear manner over development (Shaw et al., 2008). Finally, the four studies used relatively small sample sizes and different techniques for cortical morphology analysis, which may have led to varied sensitivities in the detection of the grey-matter and white matter surface.

Table 2 provides a summary of human cortical morphology research in FASD. As shown, great heterogeneity exists between studies in terms of the diagnostic site, sample size, age range and distribution, techniques, which may have contributed to the disparity in results. Further, all three cortical measures have not been investigated in any study to date.
<table>
<thead>
<tr>
<th>Recruitment Site</th>
<th>Sowell et al., 2002</th>
<th>Sowell et al., 2008</th>
<th>Yang et al., 2011</th>
<th>Fernandez-Jaen et al., 2011</th>
<th>Zhou et al., 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient sample size</td>
<td>San Diego</td>
<td>San Diego</td>
<td>San Diego, Los Angeles, South Africa</td>
<td>Spain</td>
<td>Edmonton, Canada</td>
</tr>
<tr>
<td>Age Range (years)</td>
<td>21</td>
<td>21</td>
<td>69</td>
<td>20</td>
<td>33</td>
</tr>
<tr>
<td>Diagnoses</td>
<td>8-22 (60% 8-12 yrs.; 40% 12-22 yrs)</td>
<td>8-22 (60% 8-12 yrs.; 40% 12-22 yrs)</td>
<td>8-16</td>
<td>7-16</td>
<td>6-30</td>
</tr>
<tr>
<td>Diagnoses</td>
<td>67% FAS; 33% with no facial deficits</td>
<td>67% FAS; 33% with no facial deficits</td>
<td>61% FAS; 39% not formally diagnosed</td>
<td>100% FAS</td>
<td>9% FAS; 6% pFAS; 36% neurobehavioural disorder; 48% more mildly affected cases</td>
</tr>
<tr>
<td>Technique</td>
<td>Local pipeline</td>
<td>Local pipeline</td>
<td>FreeSurfer 4.0.5.</td>
<td>Brain Voyager QX</td>
<td>CIVET 1.1.9</td>
</tr>
<tr>
<td>Cortical Thickness</td>
<td>not analyzed</td>
<td>Increased in bilateral temporal, bilateral inferior parietal, and right dorsolateral prefrontal regions</td>
<td>Increased in bilateral inferior frontal, superior temporal, inferior temporal, right middle temporal; lateral occipital, pericalcarine, postcentral and left inferior parietal, right lingual gyrus</td>
<td>Increased in bilateral temporal lobes, and Right frontal</td>
<td>Reduced in bilateral middle frontal lobes, bilateral pre- and post- central gyr, bilateral superior parietal lobe, left lateral temporal lobe, bilateral inferior temporal lobe, and bilateral occipital lobes</td>
</tr>
<tr>
<td>Surface Area</td>
<td>Reduced surface extent in the orbitofrontal region</td>
<td>not analyzed</td>
<td>not analyzed</td>
<td>not analyzed</td>
<td>not analyzed</td>
</tr>
<tr>
<td>Gyrification</td>
<td>not analyzed</td>
<td>not analyzed</td>
<td>not analyzed</td>
<td>not analyzed</td>
<td>not analyzed</td>
</tr>
</tbody>
</table>
Chapter 3
Study Questions and Hypotheses

Objective

The main objective of this thesis is to compare cortical volume determinants (thickness, surface area) as well as gyrification in children diagnosed with alcohol-related neurodevelopmental disorder (ARND) and controls. This objective will be examined in the context of three questions regarding (i) whether children with ARND show global cortical volume reductions, (ii) whether cortical volume reductions in ARND reflect cortical thickness abnormalities, and finally (iii) if cortical volume reductions in ARND emerge from abnormalities in surface area and gyrification.

Two secondary questions concern whether factors contributing to cortical development, namely age and sex, underlie cortical morphology differences between children with ARND and controls. These questions will be analyzed in Chapters 5.2.4 and 5.2.5.

The current thesis improves upon previous studies in several ways: First, it accounts for the methodological differences among prior studies of cortical thickness in FASD by using a larger sample and restricted age range and diagnosis. Second, it addresses the paucity of information on surface and gyrification in fetal alcohol affected children. Third, it examines cortical morphology in the largest known sample of relatively homogeneous non-dsymorphic patients with ARND, a prevalent yet relatively understudied subgroup within the fetal alcohol spectrum. Finally, the current study investigates age and sex effects, which are factors related to cortical development but have not been studied extensively in this clinical condition.
Question 1: Do children with ARND show reductions in global brain volumes?

Accumulating evidence suggests that individuals with FASD exhibit reductions in total brain volume (Mattson et al., 1992; Archibald et al., 2001; Lebel et al., 2008; Willoughby et al., 2008; Norman et al., 2009). These findings are consistent with those showing reduced grey matter, especially in frontal, temporal, and parietal regions (Archibald et al., 2001; Sowell, Thompson, Mattson, et al., 2002). However, limited research exists on total brain volumes exclusively in the ARND group. Therefore, the first objective of this study is to determine whether children with ARND do show global brain volume reductions relative to peers.

Hypothesis 1

Consistent with past studies, children diagnosed with ARND will show reduced brain volumes, reflecting significant reductions in cortical grey matter volumes.

Question 2: Do cortical volume reductions in ARND reflect cortical thickness abnormalities?

Past studies of cortical thickness in FASD have yielded varying results. The three papers by Sowell et al. (2008), Yang et al. (2011), and Fernandez-Jaen et al. (2011) all report increased cortical thickness in large areas of temporal, parietal, and frontal regions, whereas the one study by Zhou et al. (2011) reports cortical thinning. Sowell (2008) noted that in 7 non-dysmorphic patients with PAE, fewer areas of cortical thickening were observed and they were less affected than their FAS counterparts. However, we do not know how cortical thickness varies across subgroups such as ARND. Therefore, the aim is to determine whether
children with ARND show abnormalities of cortical thickness. The sample in the study by Zhou et al. (2011) was comprised mostly of mildly affected individuals who showed no facial abnormalities. The Zhou et al. (2011) study also used the same Canadian diagnostic approach and technique for analyzing cortical morphology (CIVET) as the current study. As such, of the four studies, the cortical thinning observed in Zhou et al. (2011) best predicts the outcome of the current study and is more reflective of the characteristics of our sample.

**Hypothesis 2**

Based on the Zhou et al. (2011) study showing cortical thinning of superior and middle frontal, superior parietal, inferior temporal, and occipital regions in mildly affected individuals within the fetal alcohol spectrum, children diagnosed with ARND will show reduced cortical thickness in similar regions.

**Question 3: Do cortical volume reductions in ARND reflect surface area and gyrification abnormalities?**

Although limited evidence exists on the surface area features of children with FASD, human autopsy studies reveal less gyrification of the cortex in individuals exposed to alcohol prenatally (Clarren et al., 1978; Wisniewski et al., 1983; Konovalov et al., 1997; Roebuck et al., 1998). Furthermore, one human neuroimaging study has shown that the brain surface extent is reduced significantly in individuals with FASD (Sowell et al., 2002a). However, as research on surface area and gyrification indices in children with ARND is lacking, a further goal will be to determine whether children with ARND show abnormalities in surface area and its closely related factor of gyrification.
**Hypothesis 3**

Based on the studies showing reduced gyrification in FASD and the findings of smaller surface extent, it is hypothesized that children diagnosed with ARND will show reduced surface area and gyrification.

**Secondary Question 1: Does the developmental trajectory of cortical measures in ARND reflect that of typically developing controls?**

Age-related trajectories of cortical thickness, surface area, and gyrification have not been investigated extensively in FASD. The cross-sectional study by Zhou et al. (2011) showed that individuals with FASD undergo similar cortical thinning as do controls in the age range from 6 to 30 years; however, these changes were manifested in different brain regions within the two groups. In another population (viz., ADHD), Shaw et al. (2007) showed that cortical thickness development followed a curvilinear pattern characterized by a delay in the peak of attaining maximum cortical thickness in these patients. However, to date no longitudinal or cross-sectional studies have analyzed age-related trajectories of cortical measures in ARND. Therefore, a further aim of this study will be to examine age trajectories in cortical measures in ARND in relation to those of normal controls. This issue will be examined by plotting the various cortical measures as a function of developmental age and group.

**Hypothesis 4**

In view of the cross-sectional data on age-trajectories in FASD and the fact that ARND is often comorbid with ADHD, it is hypothesized that ARND and controls will show similar age-trajectories on cortical measures reflecting a preadolescence increase followed by a post-
adolescence decrease; However, the decline will occur slightly later in ARND as observed in ADHD

Secondary Question 2: Do ARND participants show sexual dimorphism in cortical outcomes following prenatal alcohol exposure?

A paucity of research exists on sexual dimorphism in individuals with FASD despite clear animal evidence showing sexual dimorphisms in stress responsiveness and other behavioural indices (Weinberg et al., 2008). These dimorphisms typically reflect the greater vulnerability of alcohol-exposed female than male rodents to short and acute stressors. However, the only human FASD study to date investigating sex differences in cortical morphology showed a group by sex interaction in cortical volumes reflecting adult males with FASD being more affected than females with FASD (Chen et al., 2011). Thus, further research is needed to clarify these conflicting findings.

Hypothesis 5

Based on past research showing sex differences in the response to prenatal alcohol exposure, it is hypothesized that a group by sex interaction will be seen in cortical measures reflecting more marked sexual dimorphism in the ARND than the control group. However, due mixed results from past studies, it cannot be determined as to whether males or females will be more or less significantly affected.
Chapter 4
Methods

4.1 Participants

Participants included 121 children (mean age = 11.6 years ± 1.9; age range = 8.1-16.4 years; 55% male), 57 diagnosed with ARND and 64 typically developing controls, all of whom received MRI scans as part of four different study protocols. All participants were initially screened for lack of head injury, or a debilitating or chronic medical condition. They were also screened for MRI contraindications such as braces and metal implants. Parents or caregivers provided written informed consent and participants assented to participation in the study. The procedures for each of the four study protocols were independently approved by the Research Ethics Board of the Hospital for Sick Children (Toronto, Canada).

The clinical group consisted of participants previously diagnosed with ARND (mean age = 12.0 years; age range = 8.1-16.4 years) at the Motherisk Follow-up Clinic at the Hospital for Sick Children. Thirty-two were male and twenty-five were female. This clinic serves as a diagnostic facility for FAS or ARND and uses the Canadian guideline system (Chudley et al., 2005). To have received a diagnosis along the fetal alcohol spectrum, children had to have a documented history of heavy prenatal alcohol exposure substantiated by (i) maternal verification of alcohol related diagnosis/treatment during pregnancy, (ii) removal from mother by the Children’s Aid Society (CAS) due to her alcohol abuse or heavy drinking during pregnancy, or (iii) other sources (e.g., adoption records). In most cases, exposure documentation was acquired through CAS foster or adoption records or in statements made by the biological mother or a relative. Most children attending the clinic were accompanied
by foster parents, adoptive parents, or CAS caseworkers, while a minority came with a biological parent or relative (usually a grandparent). All assessments were made by qualified staff that included: (i) a board certified pediatrician trained in FAS diagnosis who performed neurological and physical assessments and facial dysmorphology evaluations; and (ii) a registered psychologist, psychometrist, and speech therapist, who performed different aspects of the neuropsychological evaluation. To receive an ARND diagnosis, children had to show a minimum of three significant areas of deficit, consistent with the Canadian guidelines for FASD diagnosis (see Chudley et al., 2005). In cases where the patients had undergone a psychological assessment elsewhere within one year of the diagnostic referral, the Motherisk Clinic supplemented existing assessments with additional tests.

Control participants were typically developing children (mean age = 11.3 years; age range = 8.2-15.6 years) with 34 males and 30 females. They were recruited either through community and hospital postings or were biological children of a participating adoptive or foster parent. None had a documented history of PAE or exposure to other teratogenic substances, history of learning disabilities, a known neurological or psychiatric condition.

In the control group, the majority of participants were Caucasian (74%) while other ethnicities included Mixed Race (6%), Native (2%), and 19% were not identified. The FASD group differed in composition with a sample consisting of 38% Caucasian, 20% Native, 11% Mixed Race, 7% Asian, 2% Black, and 22% Unspecified.

### 4.2 Tests and Measures

Parents or caregivers of controls and patients completed a comprehensive child history form that sought detailed information about the child’s prenatal, birth, and developmental history as well child’s age, sex, ethnicity, and family occupations. Socioeconomic status was
computed using the Hollingshead Four-Factor Index (Hollingshead, 1975) based on the education and occupation of biological or foster parents. The SES score represented an average SES score of both parents or in some cases, a single parent from current adoptive, foster, or biological family. Participants were also assessed for intelligence using the Wechsler Abbreviated Scale of Intelligence (WASI; Wechsler, 1999), which provides a Full IQ score.

### 4.3 Image Acquisition and Processing

High-resolution research-acquired T1-weighted magnetic resonance imaging (MRI) scans were obtained for each participant using a 1.5 Tesla GE scanner (General Electric Medical Systems, Milwaukee, Wisconsin). For three studies (N = 88, mean age = 11.2 years, age range = 8.1-15.6 years), scans were taken in the axial plane (TR=10.06ms, TE=4.2ms, TI=400ms, flip angle=20°, FOV=180mm, Acquisition Matrix=256x192, slice thickness=1.5mm). In the fourth study (N = 33, mean age = 12.9, age range = 9.3-16.4 years), scans were acquired in the sagittal plane (TR= 8.4ms, TE=4.2ms, TI=400ms, flip angle=15°, FOV=180mm, Acquisition Matrix=256x256, slice thickness=1.0mm). A manual quality check was conducted to assess to ensure there was no movement issues.

All scans were processed using the automated CIVET pipeline (version 1.1.10; Montreal Neurological Institute at McGill University, Montreal, Quebec, Canada). First, scans were registered to the symmetric ICBM 152 template (Collins et al. 1994). The RF inhomogeneity was corrected (Sled et al. 1998), skulls were stripped (Smith 2002), and tissue was classified into grey matter, white matter, and cerebrospinal fluid (CSF) (Zijdenbos et al. 2002; Tohkha et al. 2004). Next, deformable models were used to construct for both the right and left hemispheres, the inner white matter surface and grey matter-CSF interface or pial surface
(Kim et al. 2005); this yielded four surfaces of 40,962 vertex points each. At the next step, cortical thickness was measured from each vertex point on the white matter surface to the corresponding point on pial surface (Lerch and Evans 2005). The cortical thickness data were blurred with a 20-mm surface-based diffusion blurring kernel (Chung and Taylor 2005) and non-linearly aligned using surface based registration (Lyttelton et al 2007). Surface area was also computed at each vertex point of the pial surface by estimating the two dimensional area of a triangle formed by three vertices on the surface mesh and attributing a third of this area to each of the three vertices (Lyttelton et al. 2009).

In addition to the vertex-wise analysis, each cortical hemisphere was segmented into sub-regions using the automatic non-linear image matching and anatomical labeling (ANIMAL) algorithm (Collins et al., 1995). From these data, measures of total cortical grey matter volume, total surface area, and average cortical thickness were derived for each of the lobes (frontal, parietal, temporal, occipital).

Gyrification was computed at each vertex using the surface ratio (Toro et al., 2008a). The surface ratio is the total surface 3-dimensional area of the cortex within a sphere of a given radius divided by the two-dimensional area of a disc of that radius. These analyses were conducted using radii of 20 mm, which accounts for two or more sulci while maintaining high resolution and has been recommended for use in clinical studies (Schaer et al., 2008). A global measure of gyrification was obtained by obtaining the mean surface ratio at 20 mm for each hemisphere.

4.4 Statistical Analysis

For all vertex-wise analyses, the RMINC package (version 1.0) was used for statistical analysis. Demographic measures and lobe-wise and hemispheric analyses were conducted
using IBM SPSS Statistics 19.0 for Macintosh. Effect sizes were computed using Cohen’s $d$, which signifies a small effect for values between 0.2 and 0.3, a medium effect for values around 0.5, and a large effect for values between 0.8 and infinity. All analyses were covaried for age, sex, imaging acquisition protocol, and handedness.

In terms of categorical demographic measures, chi-squared tests were used to examine group differences in sex and handedness. For continuous measures such as age, group differences were examined using t-tests.

Group differences in cortical thickness, surface area, and the gyrification index/surface ratio were investigated at each vertex point using a general linear model. Results were corrected for multiple comparisons using a False Discovery Rate (FDR) of 5%, where $q < 0.05$ was significant (Genovese et al. 2002). This whole-brain minimally-biased approach was employed because prenatal alcohol exposure has been shown to disrupt the arealization process that defines the lobar boundaries.

For examining group differences in hemispheric gyrification and lobe-wise measures of cortical grey matter volume, white matter volume, surface area, and average cortical thickness, a general linear model was used while controlling for age, sex, acquisition protocol, and handedness. In order to eliminate variance associated with the global effects of prenatal alcohol exposure, cortical volume comparisons were corrected for total brain volume and surface area analyses were covaried for total surface area. To account for multiple comparisons for the eight lobar regions, lobe-wise results were corrected using a Bonferonni-adjusted alpha level of 0.006 per test ($0.05/8$). Analyses at the hemispheric level were corrected using the Bonferonni adjusted alpha level of $0.0025$ per test ($0.05/2$), which accounts for comparisons of two hemispheres.
In order to analyze the effects of age, gender, and hemisphere as well as age-by-group, sex-by-group interactions, we used general linear models with the factors described above as independent and cortical feature measures as dependent variables.
Chapter 5
Results

5.1 Demographic and Behavioural Data

Table 1 shows demographic data for ARND and control groups. Groups did not differ in age ($t(119) = -1.87, P = 0.06$), handedness ($\chi^2=1.3, P = 0.52$), or sex ($\chi^2=0.11, P = 0.74$).

However, ARND participants had a significantly lower mean IQ ($M = 87.1, SD = 14.8$) than controls ($M = 116.1, SD = 11.9$), $t(119) = 10.43, P < 0.001$. Full-Scale IQ ranged from 56-116 in the ARND group and 75-143 in the control group.

ARND participants also had a significantly lower SES score ($M = 2.64, SD = 1.18$) than normal controls ($M = 1.77, SD = 0.85$), indicating lower socioeconomic status, $t(119) = -4.75, P < 0.001$. In the ARND group, 42% had High/Medium High (Hollingshead scores of 1 or 2) while 33% had Medium (score of 3) and 23% had Medium Low/Low (scores of 4 or 5) SES in contrast to 83%, 13%, and 5% of controls respectively.

All control cases were living with at least one biological parent, whereas only 18% of ARND participants were in biological care, with 19% reported to be in foster care and 63% were adopted.

As shown in the Table 3, ARND participants had prenatal exposure to other drugs and 60% of ARND patients showed a ADHD comorbidity, whereas controls were less affected by these factors.
<table>
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<th>Controls</th>
<th>p value</th>
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<td>116.1(11.9)</td>
<td>p &lt; 0.001</td>
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5.2 Neuroimaging Results

5.2.1 Question 1: Reduced Brain Volumes

Total brain volumes were significantly smaller in the ARND group (M = 1303.61 cm$^3$, SD = 152.59 cm$^3$) as compared with the control group (M = 1383.01 cm$^3$, SD = 132.49 cm$^3$), $F = 12.52$, $P = 0.001$, Cohen’s $d = 0.60$. Group effects in total brain volume remained significant even when ventricles were excluded from the analysis, $F = 10.23$, $P = 0.002$, Cohen’s $d = 0.52$.

Total grey matter volumes were also significantly smaller in the ARND group (M = 627.48 cm$^3$, SD = 77.94 cm$^3$) as compared with controls (M = 670.84 cm$^3$, SD = 70.24 cm$^3$), $F = 10.43$, $P = 0.002$, Cohen’s $d = 0.62$. Results uncorrected for total brain volume showed a significant reduction of absolute volumes of left and right frontal ($P = 0.002$ for both), left and right parietal ($P = 0.006$, $P < 0.001$), and right temporal grey matter ($P = 0.001$) cortical volumes in ARND (see Figure 5). There was a significant interaction between lobe and group ($F = 11.91$, $P = 0.001$). None of the lobar cortical volume differences remained significant after controlling for total brain volume.

White matter volumes were also significantly lower in the ARND group (M = 366.37 cm$^3$, SD = 64.84 cm$^3$) compared with controls (M = 375.19 cm$^3$, SD = 481.76 mm$^3$), $F = 4.84$, $P = 0.030$, Cohen’s $d = 0.18$. However, no regional reductions of white matter were observed.

In addition, the lateral ventricles were significantly smaller in the ARND group (M = 102.14 cm$^3$, SD = 26.92 cm$^3$) versus controls (M = 115.57 cm$^3$, SD = 26.33 cm$^3$), $F = 4.48$, $P = 0.037$, Cohen’s $d = 0.51$. These reductions were no longer significant when controlling for total brain volume, suggesting a global reduction.
Finally, the fourth ventricle was significantly smaller in the ARND group (M = 1.88 cm$^3$, SD = 0.55 cm$^3$) as compared with controls (M = 2.25 cm$^3$, SD = 0.70 cm$^3$), $F = 8.24$, $P = 0.005$, Cohen’s $d = 0.54$. In contrast to the other structures, this group effect remained robust even when controlling for total brain volume, $F = 5.16$, $P = 0.025$. 
Figure 5 Adjusted group differences in lobar cortical grey matter volumes (cm$^3$) after controlling for age, sex, acquisition protocol, and handedness (* = significant group difference at p < 0.006). Effect sizes (Cohen’s d) are indicated above the bars.
5.2.2 Question 2: No Cortical Thickness Differences

Vertex-wise and lobe-wise analyses on uncorrected data as well as data corrected for multiple comparisons at 5% FDR showed no significant group differences in cortical thickness within left or right hemispheres. See Figure 6 for the cortical thickness plots. Cortical thickness analyses were conducted on the total sample, within gender groups and acquisition protocol groups.

5.2.3 Question 3: Reduced Surface Area and Gyrification

Global and Local Surface Area Reductions

Total surface area was significantly reduced in the ARND group (M = 181.60 cm$^2$, SD = 18.80 cm$^2$) relative to controls (M = 188.73 cm$^2$, SD = 15.28 cm$^2$), $F = 13.93$, $P < 0.001$ Cohen’s $d = 0.38$. Results uncorrected for total SA revealed significant reductions of absolute surface area of left and right frontal ($P = 0.001$ for both) and left and right temporal lobes ($P < 0.001$ for both) (see Figure 7) However, when total surface area was controlled for, only the right temporal lobe SA approached significance, ($F = 7.17$, $P = 0.009$, Cohen’s $d = 0.48$; adjusted mean difference = 934.398 mm$^2$). Further, vertex-wise analyses revealed these SA abnormalities were confined to the right superior temporal gyrus and a region between the right temporal and occipital cortices, $t(119) = -2.73$, $q < 0.05$ (see Figure 8).
Adjusted group differences in mean cortical thickness (mm) after controlling for age, sex, acquisition protocol, and handedness.
Figure 7 Adjusted group differences in lobar surface area (cm$^2$) after controlling for age, sex, acquisition protocol, and handedness (* = significant group difference at $p < 0.006$). Effect sizes (Cohen’s d) are indicated above the bars.
Figure 8 Right hemisphere brain map portraying t values of group comparisons after correcting for multiple comparisons using FDR (q < 0.05). The blue colours indicate areas of significant surface area reductions in ARND.
Global Gyrification Reductions

The vertex-wise surface-ratio analysis suggested global reductions in gyrification. The mean surface ratio at radius of 20 mm revealed significant reductions in the left hemisphere, $F = 8.18, P = 0.005$, Cohen’s $d = 0.46$ and right hemisphere, $F = 9.01, P = 0.003$, Cohen’s $d = 0.49$ (See Figure 9). However, no local gyrification abnormalities detected at $radius = 20\text{mm}$ from each vertex point.
Figure 9 Group differences in mean hemispheric surface ratio (surface in sphere of radius 20 mm/area of disc with same radius) after controlling of age, gender, acquisition protocol, and handedness (* = significant group difference at p < .025)
5.2.4 Secondary Questions 1: Age Trajectories

No significant age by group interactions were found on measures of cortical thickness or gyrification at both the hemispheric and lobar levels. However, qualitative analysis of graphs on the lobar level values showed trends for surface area indices with peaking occurring earlier in all brain regions in typically developing children than those with ARND (see Figure 10). Trend-level interactions of age and group interactions were observed for surface area of the left frontal region, $F = 4.41, P = 0.038$, and the right occipital region, $F = 4.28, P = 0.041$.

5.2.5 Secondary Questions 2: Sex Dimorphism

Males and females did not differ in age ($t(119) = 1.24, P = 0.22$) or handedness ($\chi^2 = 1.38, P = 0.50$). However, results revealed a significant interaction of group and sex on total brain volumes, $F = 4.53, P = 0.036$, $R^2 = 0.33$, with females in the ARND group showing much larger reductions in brain volume versus males (see Figure 11). Consistent with this finding, also noted was a significant group by sex interaction for cortical grey matter volumes, $F = 4.02, P = 0.048$, $R^2 = 0.30$, reflecting the greater cortical grey matter volume reductions in females with ARND than males with ARND (see Figure 12). As shown in Figure 13, lobe-wise analyses indicated that females were more affected than males in terms of cortical volume reductions in the temporal and parietal cortices. Finally, the cortical volume reductions observed were driven more by sexual dimorphism in surface area (see Figure 14) than cortical thickness or gyrification (data not shown), for which no significant group by sex interactions were observed.
Figure 10 Age-related trajectories in surface area (cm$^2$) by brain region. The blue lines follow the trajectory for typically developing controls while the red line represents the ARND group.
Figure 11 Sexual dimorphism results showing that females in the ARND group are affected more than males in terms of total brain volume (cm$^3$) reductions. (* = significant group difference at p < .001)
Figure 12 Sexual dimorphism results showing that females in the ARND group are affected more than males in terms of cortical grey matter volume (cm$^3$) reductions. (* = significant group difference at $p < .001$)
Figure 13 Sexual dimorphism results showing that in the ARND group, females are affected more than males in terms of cortical grey matter volume (cm$^3$) reductions, particularly in the parietal and temporal regions. (* = significant group difference at $p < .001$)
Figure 14 Sexual dimorphism results showing that females in the ARND group are affected more than males in terms of surface area (cm²) reductions. (* = significant group difference at $p < .01$)
5.2.6 Sub-analysis: ADHD Comorbidity

Because 60% of the sample had ADHD, which is also associated with cortical abnormalities, a sub-analysis was conducted to evaluate if ARND groups with ($n = 34$) and without ADHD ($n = 23$) differed in terms of brain measures.

The ARND groups with and without ADHD did not differ significantly from each other in terms of total brain volumes (see Figure 15A), cortical volumes (see Figure 15B), and total surface area (see Figure 15C), $P > 0.05$. Consistent with these results, the ARND groups with and without an ADHD comorbidity did not differ in mean Full-Scale IQ.

Lobe-wise and vertex-wise analysis further revealed no significant difference between the ARND groups with and without an ADHD comorbidity in terms of local surface area reductions of the right temporal lobe (data not shown).
Figure 15 Mean values for A) total brain volume (cm$^3$) B) total cortical volume (cm$^2$) and C) total surface area (cm$^2$) in ARND groups with and without an ADHD comorbidity.
Chapter 6  
Discussion

The current study aimed to compare cortical volumes and their determinants in children diagnosed with alcohol-related neurodevelopmental disorder (ARND) and controls. This was conducted in the context of three specific questions concerning (i) whether children with ARND show global cortical volume reductions, (ii) whether cortical volume reductions in ARND reflect cortical thickness abnormalities, and finally (iii) if cortical volume reductions in ARND emerge from abnormalities in surface area and gyrification. Two supplementary questions regarding the effects of age and sex on measures of cortical morphology were additionally explored.

The main results showed global total brain volume reductions as well as cortical volume reductions in frontal, parietal, temporal regions in children with ARND. These volume reductions did not reflect cortical thickness abnormalities as no significant group differences were observed on this index. Nevertheless, the ARND group did show significant global reductions in cortical surface area. After controlling for global effects, reductions in surface area of the right temporal lobe approached significance. Vertex-wise analyses also revealed that these surface area reductions were confined to the right superior temporal gyrus and the right occipital-temporal area. Consistent with surface area findings, significant global reductions in gyrification of both hemispheres were found.

Supplementary questions examined whether group differences in cortical volumes were driven by dissociations in the age-trajectories or by differences in sex. Although no significant group by age interactions were observed on indices of cortical thickness and gyrification, cross-sectional plots of surface area over developmental age showed curvilinear
trends with the ARND group peaking later than typically developing controls in the surface areas of many brain regions. In addition, significant group by sex interactions were observed for total brain volumes and for cortical volumes in the temporal and parietal regions, with females showing greater vulnerability to the teratogenic effects of alcohol as compared with males. This sexual dimorphism in global cortical volume reductions in ARND reflected sex differences in surface area and not cortical thickness or gyrification.

6.1 Global Brain Volume Reductions

Total Brain Volumes

The findings of reduced global brain volumes are consistent with early post-mortem studies (Clarren and Smith, 1978; Jones and Smith, 1973; Jones et al., 1973; Wisniewski et al., 1983), as well as with more recent MRI research (Archibald et al., 2011; Mattson et al., 1996; Willoughby et al., 2008) showing that prenatal alcohol exposure leads to reduction in total brain volumes.

In line with the findings of the current study, past FASD research has also shown global reductions in frontal, temporal, and parietal cortical grey matter volumes; however these group effects do not remain significant when total brain volume is controlled for (Archibald et al. 2001; Mattson et al. 1994; Lebel et al., 2008; Bjorkquist et al. 2010). The results therefore imply a global effect of prenatal alcohol exposure on the developing brain.

One mechanism that may be responsible for these effects is alcohol’s induction of excessive neuronal cell death. Indeed, extensive research on rodent models of FASD has shown neuronal cell death occurs as a consequence of oxidative stress and production of free radicals (Bredesen, 1996) as well as excitotoxicity (Michaelis and Michaelis, 1994) in
ethanol exposed rodent fetuses. Alcohol may also inhibit neuronal proliferation by adversely affecting the cell division process (Mikami et al., 1997), particularly in early stages of embryogenesis.

Another potential cause of these global reductions is alcohol’s interaction with some of the fundamental genes that govern brain size such as microcephalin (MCPH1), CDK5RAP2 (MCPH3), ASPM (MCPH5), and CENPJ (MCPH6). Recent research has implicated MCPH genes in primary microcephaly, a disorder characterized by having a small head circumference that is one-third of the normal size (Rimol et al., 2010). Additionally, MCPH genes are expressed in the neuroepithelium of the ventricular zone and serve to mediate the symmetric mitotic division of the progenitor cells during cortical development. Alcohol may reduce the expression of genes such as MCPH in the neuroepithelium of the ventricular zone and this may lead to a drastic reduction in the number of cell divisions that give rise to precursors of the developing brain.

Current findings also showed reduced global white matter in children with FASD, which is consistent with past research demonstrating that prenatal alcohol exposure reduces levels of myelin basic protein, thereby leading to reduced white matter volumes and decreased myelination (Archibald et al., 2001).

**Ventricular Volumes**

In contrast to past research showing enlarged lateral ventricles in animal models (Godin et al., 2010) and humans (Swayze et al., 1997), the current study found reduced lateral ventricle volumes but these did not remain significant when total brain volume was considered. However, previous studies have investigated ventricular volumes only in dysmorphic cases,
who were likely to be more sensitive to alcohol early in embryogenesis when the ventricles are developing. In comparison, the participants in the current study were non-dysmorphic and may have had different genetic susceptibilities to the alcohol exposure or may have been exposed to alcohol at a different time point. Indeed, animal studies have shown that timing of alcohol exposure has a profound impact on brain and facial phenotypes with lateral ventricles being affected greatly if exposure occurred on day 17 of pregnancy, but not later on day 21 (Parnell et al., 2009; Godin et al., 2010).

A significant reduction of the fourth ventricle beyond total brain volumes was also found. This is inconsistent with past findings of enlarged fourth ventricles (Parnell et al., 2009). However, once again, these findings stem from studies on dysmorphic animals that were exposed to alcohol very early in embryogenesis and thus these findings cannot be generalized to other subtypes of FASD, including ARND.

6.2 No Significant Cortical Thickness Differences

The results for cortical thickness differences in individuals with FASD in the literature are inconsistent showing either cortical thickening (Sowell et al. 2008; Yang et al. 2011; Fernandez-Jaen et al. 2011) or cortical thinning (Zhou et al. 2011) in areas of the frontal, temporal, and parietal cortices. The current results of no significant cortical thickness differences between ARND and control groups add to this discrepancy.

The conflicting results among past studies and current observations may be due to several factors: First, the various studies consisted of different clinical groups of participants, who varied in terms of diagnoses, age ranges, and levels and timing of exposure to alcohol. The past studies also used less stringent criteria for diagnosis, recruited participants from multiple sites, and included a portion of the sample that had not been formally diagnosed (i.e. Yang et
al., 2011). In comparison, the current study eliminated this variability by focusing strictly on a sample of participants diagnosed with ARND. Furthermore, all clinical participants received a diagnosis at the same site using the more sensitive Canadian diagnostic system, which requires a child to have at least three significant areas of neurobehavioural deficits to be given a diagnosis of ARND (Chudley et al., 2005). Thus, it is possible that the findings of no significant cortical thickness abnormalities may be unique to the ARND subtype.

Furthermore, whereas some of the previous studies involved patients with a broad age range extending as high as age 30 (Zhou et al., 2011), the current study investigated a narrower age range from 8 to 16 years. The results are therefore indicative of cortical measures in the ARND clinical group at a circumscribed developmental stage that includes only pre- and early adolescence. This is critical in the interpretation of results because cortical thickness has been shown to vary developmentally in a curvilinear manner during adolescence with the peak increase corresponding to the timing of puberty, which is followed by a decrease (Shaw et al., 2008). Thus, depending on the age-range analyzed, the results could be skewed towards trends of cortical thickening, thinning, or no change at the peak of maximum cortical thickness. For instance, in the study by Sowell et al. (2008), cortical thickness was investigated in sample composed of 60% of individuals between the ages of 8-12 years. During this period of development, abnormalities in the remodeling of the ontogenetic columns or anomalies such as heterotopias may manifest as increased cortical thickness as shown in the aforementioned studies. In contrast, the study by Zhou et al. (2011) investigated a sample over a much broader age-range (6-30 years). At this time, abnormal synaptic pruning (Huttenlocher and Dabholkar, 1997) may result in decreased cortical thickness as found in the study by Zhou et al. (2011).
Other important factors to consider are methodological differences between studies such as their different image processing pipelines and protocols for correcting for multiple comparisons. Such variations may have led to varied sensitivities in detecting cortical features. The current study used the CIVET pipeline to analyze cortical morphology and corrected for multiple comparisons using a relatively stringent criterion, namely the False Discovery Rate (FDR) set at 5% (Genovese et al., 2002). In contrast, past studies used other processing pipelines (see Table 2), different correction methods (Sowell et al., 2008; Yang et al., 2011), and in one case, did not account for multiple comparisons (Fernandez-Jaen et al., 2011). Because cortical thickness is a very minute measure analyzed over thousands of points across the cortical surface, failure to properly correct for multiple comparisons could lead to Type 1 error, resulting in false positive results.

Finally, the lack of cortical thickness findings in the current study may be due to issues of scan quality. The cortical thickness findings are only as accurate as the spatial resolution and $T_1$ contrast of the MRI scans, which depend on the field strengths and other properties inherent to the scanner. Higher field strengths and better quality imaging technologies yield a greater signal-to-noise ratio, and better contrasts between white matter, grey matter, and cerebrospinal fluid. This enables better calculation of true cortical thickness distance ($t_{\text{link}}$), which depends on precise identification of the grey matter-white matter interface (Fischl and Dale, 2000). Any issues with the grey/white matter contrast of the scans could have skewed this boundary, leading to erroneous estimates of cortical features.

### 6.3 Surface Area and Gyrification Reductions

#### Global Surface Area
Children with ARND showed reduced global surface area, which is consistent with one past study showing that the average brain surface extent is smaller in individuals exposed prenatally to alcohol as compared with typically developing controls (Sowell et al., 2002a). The current finding of reductions in surface area but not cortical thickness is in line with past research showing a dissociation between these measures in terms of genetic polymorphisms (Panizzoni et al. 2009), cellular mechanisms, and developmental stages (Rakic 1995).

It is well established that distinct genetic mechanisms underlie surface area and thickness. During human evolution, a large increase was observed in the surface area of the cortex with very little change in cortical thickness, providing evidence for dissociation between the two mechanisms (Northcutt and Kass, 1995). Panizzoni et al. (2009) further showed that the genes underlying surface area and cortical thickness are not correlated, suggesting independent mechanisms. Recent research has confirmed this finding showing that genes such as MCPH 1-7 (Rimol et al., 2010), MECP2 (Joyner et al., 2009), and KTCD8 (Paus et al., 2010) profoundly affect surface area, but not cortical thickness. Thus, if alcohol has epigenetic effects on the genes underlying corticogenesis, it is very possible that a disruption of global surface area occurs without much change in cortical thickness.

On the cellular level, surface area is hypothesized to stem from symmetrical division of progenitor cells in the ventricular zone during the first six weeks embryogenesis (Chenn and Walsh 2002), which establishes the founders of the ontogenetic radial columns that define the area magnitude (Mountcastle, 1997). In contrast, cortical thickness is hypothesized to result from the asymmetric division of the progenitor cells that yield postmitotic neurons, which in turn build the later developing cortical columns (Rakic, 1995). Thus, if prenatal
alcohol exposure occurs early in gestation as is the case with most unplanned pregnancies, this may disrupt surface area to a greater extent than cortical thickness.

This disruption of surface area may occur through several mechanisms. First, apoptosis of progenitor cells due to oxidative stress, excitotoxicity, or other mechanisms during this period would lead to a significant decrease in the number of radial units that contribute to surface area. The symmetric mitotic division of progenitor cells may also be disrupted, decreasing the progenitor pool and the precursor radial units. As such, if alcohol adversely affects the mechanisms involved in the symmetric division stage, this would have a profound impact on the number of radial units and the subsequent construction of surface area.

Surface area is largely established in early development and changes very little after birth and therefore prenatal alcohol exposure likely has long-term effects on cortical surface area. On the other hand, although cortical thickness can be disrupted during the prenatal period, it is possible that this reduction is counteracted by growth in cortical layers that occurs after birth well into adolescence.

**Local Surface Area**

Beyond the total surface area reductions, local reductions were observed currently in the right temporal lobe. Analyses on a vertex-level showed that these surface area reductions were found in two main regions of the right hemisphere, namely the occipital-temporal area and the superior temporal gyrus.

Consistent with these findings, previous studies have shown structural grey matter volume reductions in the occipital-temporal area of individuals exposed prenatally to alcohol (Sowell et al., 2002a; Li et al., 2008). The occipital-temporal area is implicated in visual processing,
specifically in the recognition of object features (Beauchamp, 2005) and is strongly governed by attention processes (Kanwisher and Wokciulik, 2000). In line with this research, Li et al. (2008) observed using fMRI that individuals with prenatal alcohol exposure show abnormalities in this area when performing sustained visual attention tasks involving shape recognition. As such, occipital-temporal abnormalities in surface area may underlie deficient visual processing of object features.

The other area of interest in which a significant group effect was observed is the right superior temporal gyrus, which is one of the heteromodal association areas that develops last in terms of grey matter maturation in the cortex (Gogtay et al., 2004), particularly because this area is involved in the higher-order processes that are more recently evolved.

The superior temporal gyrus is an important component of the social cognition network (Baron-Cohen et al., 1999) and has been shown to be abnormal in individuals with autism (Jou et al., 2010), who exhibit comparable socially inappropriate behaviours as seen in FASD (Bishop et al., 2007). Indeed, Casanova et al. (2002) using postmortem data showed that the cell columns that define surface area in the posterior superior temporal gyrus were significantly smaller in cases with autism. As such, findings of surface area reductions in the superior temporal gyrus may account for the social cognition deficits observed in individuals ARND (Bishop et al., 2007; Greenbaum et al., 2009).

The right superior temporal gyrus is also involved in other integrative functions. It is located in close proximity to the auditory cortex and has been shown to be activated during auditory discrimination tasks involving detecting the timing of auditory stimuli (Bueti et al., 2008). Lesion studies further suggest that the right superior temporal gyrus is implicated in spatial attention orienting in response to gaze cues (Akiyama et al., 2006).
Both the temporal and occipital areas of the brain also peak late in cortical thickness as compared to the other lobar regions (Giedd et al., 1999). As such, the local abnormalities in the right superior temporal gyrus and occipito-temporal region may be due to alcohol exposure later in pregnancy. In fact, the population under study was not only subjected to alcohol early in development, but throughout.

**Gyrification**

In terms of the gyrification analysis, global reductions of the surface ratio were seen in the ARND group as compared to the control group. However, no local gyriﬁcation were observed at a radius of 20 mm. Current ﬁndings of reduced gyriﬁcation across the cortical surface are consistent with autopsy studies showing reduced gyriﬁcation patterns in individuals exposed to alcohol in utero (Clarren et al., 1978; Wisniewski et al., 1983; Konovalov et al., 1997; Roebuck et al., 1998).

The global reduction in gyriﬁcation may be linked to the global reduction in surface area. It is possible that apoptosis of progenitor cells early in embryogenesis not only affects surface area, but also leads to less gyriﬁcation of the smaller cortical surface. In the third trimester of human development, gyriﬁcation results from the folding of cortical area as the brain undergoes rapid expansion (Chi et al., 1997). It is hypothesized that this folding results from the tensions within cortical ﬁbres in adjacent ontogenetic columns in the developing brain (Van Essen and Drury, 1997). Thus, it follows that if surface area is less and the number of ontogenetic columns is fewer, there will be fewer interactions between the cortical ﬁbres, thus leading to reduced global gyriﬁcation. In line with this theory, several genetic
microencephaly disorders are characterized by simplified gyral patterns to more severe lissencephaly as in the case of Miller-Dieker syndrome.

The reduced global gyrification may also be related to the alcohol’s disruption of neuronal migration, which causes cells to be stranded in deep layers of the cortex. As a result, this may lead to a reduction of the depth of the sulci in the brain, showing a lower gyrification index and surface ratio. Another explanation is that the displaced neurons would not form connections with unrelated neighbouring cells, thereby creating less tension within the cortical fibres in adjacent ontogenetic columns. By consequence, this would result in a significant reduction of the folding of the cortex.

In addition, alcohol may interact with global genes governing the cortical folding process. Sporadic mutations \( L1 \) and \( DCX \) doublecortin have been linked with neurodevelopmental conditions involving lissencephaly or loss of gyrification of the cortical surface (Lo Nigro et al., 1997; Gleeson et al., 1998). These genes are implicated in microtubule-mediated migration of cells, and therefore may also affect neuronal migration during the asymmetric phase of corticogenesis.

### 6.4 Age-Related Trajectories

Exploratory analyses revealed no significant group by age interactions on cortical thickness or gyrification. Interestingly, however, the surface area graphs did show curvilinear trends with the ARND group peaking at a later age in the different lobe areas compared with typically developing controls.

The observed age-related trajectories are consistent with critical findings from previous research on brain development. First, the trends in the frontal, temporal, and parietal lobes
followed a curvilinear trajectory with a pre-adolescent increase and post-adolescent decrease in cortical surface area consistent with previous findings (Raznahan et al., 2011). Second, the age at which surface area peaked in the ARND group was approximately later that of the control group in frontal, parietal, and temporal regions.

Consistent with these findings, longitudinal investigations of children with ADHD indicate a delay in development of the cortex, specifically in terms of cortical thickness (Shaw et al. 2007). The median age at which 50% of cortical points attained their peak thickness was 10.5 years in the ADHD group versus 7.5 years in the control group. In the current study, age-trajectories for ARND were similarly delayed by three years.

Furthermore, in the ADHD study (Shaw et al., 2007), the area with the greatest dissociation in age trajectories was the prefrontal cortex with thickness reaching a peak of 10.9 years in ADHD but 5.9 years in control patients. Consistent with this result, the current study also showed a trend-level group by age interaction (uncorrected) on surface area was in the left frontal region, where the ARND patients appeared to peak significantly later in surface area than normal controls.

The possible delay in surface area development in ARND may be due to several factors. First, prenatal exposure to alcohol may alter the cortical gyrification mechanisms that increase surface area in the pre-adolescent phase of development. Second, it is possible that individuals with prenatal alcohol exposure have a delay in pubertal development and this may be reflected in their late growth in surface area and brain volume. However, no research exists on puberty in ARND. Finally, since 60% of the study sample was diagnosed with ADHD, the delayed peak in cortical surface area may be the result of this comorbidity as shown in the study by Shaw et al. (2007). However, the sub-analysis suggests that ARND
individuals without an ADHD diagnosis do not differ in terms of their surface area reductions. As such, this last explanation does not explain the developmental delay observed in the current study.

### 6.5 Sexual Dimorphism

Group by sex interactions on total brain volumes and cortical volumes observed in this study reflected the greater vulnerability of females than males to the teratogenic effects of alcohol. This effect was observed primarily for global brain reductions and was found to reflect sex differences in surface area but not cortical thickness or gyrification.

Our findings showing sexual dimorphisms in susceptibility to prenatal alcohol exposure are consistent with previous observations in animals of sex differences in stress responsiveness following exposure (Weinberg et al., 2008). Specifically, female rodents after a short duration stressor showed higher levels of cortisol than did males. In line with this finding, it is possible that the sexual dimorphism in brain volumes stem from the differential responsiveness of males and females to the alcohol-induced stress. Indeed, past studies have shown that decreased cortical volumes are associated with increased levels of cortisol (Carrion et al., 2010). Thus, the mechanism underlying the observed sexual dimorphism in cortical volumes observed presently may be attributed to the different levels of cortisol following the alcohol exposure in males and females as opposed to a direct effect of alcohol on the female versus the male brain (Weinberg, 1988).

Another explanation for the sex dimorphisms observed concerns the different male and female gonadotropic hormones, which act in utero on the developing brain (Finley and Kritzer, 1999). Previous studies have suggested that testosterone particularly protects against neuronal apoptosis in males by modulating the expression of pro- and anti-apoptotic genes
(Paus et al., 2011). If this is the case, then alcohol’s induction of progenitor cell death early in embryogenesis may be circumvented by testosterone in males and this may lead to better outcomes for males.

In contrast to past studies showing that males with ARND have more reductions of cortical volumes in many regions than do females, the current results showed that females showed greater decreases in cortical volumes as a result of alcohol exposure. The findings from another study also showed sexual dimorphisms in the corpus collosum with males being affected to a greater degree (Zimmerberg and Mickus, 1990).

The discrepancy between the current study results and past literature may be due to alcohol’s interaction with different genes that govern brain growth in sex-dependent patterns. For instance, a recent study by Rimol et al. (2010) found that the candidate genes involved in genetic microcephaly differentially affect males and females in terms of their effects on cortical features. In particular, one variant (CDK5RAP2) had effects on cortical area expansion in males, but not females, whereas another variant, MCPH1, affected only females in terms of cortical area. Similarly, another gene implicated in global brain development, KTCD8 was found to affect only females in terms of surface area and cortical folding (Paus et al. 2010).

Lastly, the current study also showed that the sex differences in cortical volume reductions were observed to be partly the result of sex dimorphisms in surface area and not cortical thickness. This pattern is line with the findings by Raznahan et al. (2011), who established the sexual dismorphism in cortical volume is an emergent property of sex differences in cortical surface area but not thickness. Moreover, this study also showed that when surface area is further delineated, the sex differences represented cortical hull area (exposed surface)
and not gyrification. Consistent with this observation, the current study did not find any group by sex interactions in gyrification.

### 6.6 Limitations

Although the results of this thesis provide critical insights into the cortical abnormalities that patients diagnosed with ARND manifest, this study has some important limitations. Like other research on individuals with FASD, it was not possible to control for confounding environmental factors such as poor pregnancy care, early life adversity, poverty, prenatal exposure to cigarettes and other drugs, stress, multiple home placements, and neglect abuse, all of which may profoundly influence the developing cortex (Abel and Hannigan, 1995; Toro et al., 2008b; Sowell et al., 2008). As shown in Table 3, the ARND group differed significantly from controls on many confounding factors. Follow-up analyses, however, have shown that the current results remained when children with a low SES of 4 and 5 were excluded.

The ethnicities of the participants also differed between groups with the proportion of Caucasian individuals being higher in the control group as compared to the clinical group. The mixed sampling of ethnicities may have led to different results than what would be expected from a more homogenous sample. For instance, certain ethnic groups may possess genetic variants (i.e. ADH1B*3) that protect their offspring from the adverse effects of alcohol exposure (Jacobson et al., 2006), leading to better neurobehavioural outcomes. However, the different prevalence rates of FASD among ethnicities is more likely due to alcohol consumption rates than genetic differences. In Canada, for example, there is a higher prevalence of FASD in Aboriginal communities where the rates of drinking are much higher.
(Masotti et al., 2006). More research is needed to determine how genetic differences and consumption rates between ethnic groups impact brain dysmorphology.

In addition, 60% of the ADHD sample were diagnosed with ADHD, which is also associated with cortical abnormalities. For instance, in the study by Fernandez-Jaen et al. (2011), it was shown that cortical thickness abnormalities are implicated in ADHD as well as FASD individuals with an ADHD comorbidity. Importantly, in their study, they showed that the FASD group was more affected than the ADHD group in terms of cortical thickening of the frontal, occipital, and right temporal areas of the brain. However, sub-analysis of our data revealed that the ARND group with the ADHD diagnosis did not differ in terms of total brain volumes, cortical volumes, and surface area on local and global levels. These findings combined with past research (Fernandez-Jaen et al., 2011) suggest that the ADHD comorbidity is not likely driving the differences in cortical morphology.

Further, the majority of typically developing participants had an IQ above 100, which is above the normative average and may indicate that the control group consists of atypical participants with above-average intelligence. This observation may partly be due to the Flynn effect (Flynn, 1987), which is a world-wise rise in IQ levels that may reflect accelerated development of younger generations. Future studies should IQ-match clinical participants with typical and atypical populations (autism, ADHD) to control for any confounding effects that a high IQ may have on cortical morphology.

Additionally, because our sample of FASD was clinic-based, information on the specific timing and precise amount of prenatal alcohol exposure was very limited. It was therefore difficult to define the exact relationship between cortical abnormalities and level of prenatal alcohol exposure. Nonetheless, high levels of alcohol exposure are suspected from
Children’s Aid Society (CAS) reports and/or relatives’ descriptions of the biological mothers’ drinking behavior throughout the pregnancy. For example, several of the participants were adopted from Eastern Europe where drinking throughout pregnancy is a known fact and now recognized to be a major problem for adoptions within European Union countries (Landgren et al., 2010). Also, grandparents and other relatives (e.g., aunts, sisters-in-law), who represent a substantial kinship group that take in a related child so as to keep the child who would otherwise be apprehended by the CAS, report seeing a heavy degree of drinking in the biological mother (typically their daughter). Finally, many of the children in the current study were fostered or adopted, often since birth due to the mothers’ known heavy drinking throughout pregnancy.

The results were not controlled for total physical growth, which may have varied slightly between groups. However, one past study has shown that group differences in cortical volume between control and alcohol-exposed groups remained significant after covarying for birth weight, birth head circumference, adult weight, and adult head circumference (Chen et al., 2011). Therefore, differences in overall physical growth should have minimal impact on the brain outcomes.

Another limitation of the current study was the variability of the MRI scans. Imaging data were derived from two different acquisition protocols among four separate investigations from which the participants were derived. As such, the scan heterogeneity may have altered the results. However, the use of acquisition method as a covariate may have compensated for this error to some degree. The images were also taken from a 1.5 Tesla scanner, which may not have provided the best signal-to-noise ratio necessary for accurately defining the boundaries between the various brain tissue types.
Finally, even though the sample size was relatively large for most FASD studies, this may have been small yielding low power for the current processing approach. The data were also examined cross-sectionally for age and so would not have provided sufficient power for examining developmental trajectories.

6.7 Future Directions

In order to account for the various limitations of this study, future research should be conducted prospectively using large samples of pregnant women who are examined for blood alcohol levels, nutritional status, and other risk factors at several time points during pregnancy. The offspring of the pregnant mothers could then be followed after birth and examined for signs of neurobehavioural impairments. This method would provide more definite evidence connecting the link between timing and degree of alcohol exposure and cortical abnormalities.

Also, in view of the findings of global cortical, surface area, and gyrification reductions in ARND, it would be of interest to investigate genetic and molecular underpinnings of these cortical abnormalities. In particular, investigation of the various interactions between prenatal alcohol exposure and genes would provide insights into the mechanisms underlying global disturbances of cortical morphology. For instance, mutations in the \textit{MCPH1} gene have been shown to lead to microencephaly with a dramatic decrease in surface area, but no effect on cortical thickness (Rimol et al., 2010). Moreover, research suggests that the \textit{MCPH1} affects cortical surface area in females and not in males, consistent with the current study results. In view of the similarities in these findings and our current results, it would be of interest to look further into alcohol’s interactions with genetic candidates such as \textit{MCPH1} that govern brain reductions.
Another area that could be explored further concerns conducting correlations between measures of structural morphology and aspects of behaviour. It is generally known that microencephalaly is linked with a reduction in IQ; however, it would be of interest to investigate global IQ deficits in relation to the development of global cortical thickness, surface area, and gyrification in ARND. A study of local cortical abnormalities in conjunction with cognitive and behavioural measures would additionally provide a more specific and biologically relevant profile for ARND. A relevant question arising from the results of this study concern whether the surface area abnormalities in the right superior temporal gyrus are linked to deficient processes such as social cognition in ARND and if the occipital-temporal regional abnormalities reflect problems with visual processing of object features.

Research is also needed to evaluate how the different indices of cortical morphology, namely thickness, surface area, and gyrification, vary within and across the various subgroups of the fetal alcohol spectrum. Future studies should include larger samples of patients from the same clinical subgroup diagnosed using the strict criteria for facial and neurobehavioural deficits. This would allow for a more accurate means of comparing different studies based on the specific population studied.

Large-scale longitudinal studies should also be conducted to delineate the age-related trajectories in the various subgroups within the fetal alcohol spectrum. This would allow for a better understanding of how development of cortical volume, cortical thickness, surface area, and gyrification is altered in this clinical population with respect to age. Ultimately, this would help inform differences in past studies that have used varying age-ranges.
Finally, it would be of interest to conduct animal studies to further examine cortical morphology in relation to different genetic susceptibility profiles. Previous research in animal models of FASD has shown that exposure to high levels of ethanol early in pregnancy results in a broad spectrum of brain and facial abnormalities (Parnell et al., 2009; Godin et al., 2010). In this study, some mice showed almost no facial abnormalities (similar to ARND), a proportion showed some facial features (similar to partial FAS), and others showed dysmorphologies consistent with full blown FAS, although all mice were genetically identical and exposed to identical amounts of alcohol on the same gestational day. This suggests that the teratogenic effect of prenatal alcohol exposure is greatly influenced by epigenetic factors (i.e. order of birth). Thus, future studies should evaluate alcohol’s epigenetic interactions with different genetic variants that can contribute to both physical and brain abnormalities in FASD. Such research will help with creating more specific and sensitive diagnostic criteria for both clinical and research purposes.

### 6.8 Implications

The global reductions in cortical volumes and surface area combined with the local effects in polymodal association areas may underlie the diffuse and global neurobehavioural abnormalities of ARND patients. This has implications for developing interventions and therapy targeted towards ameliorating brain outcomes for the ARND population.

The use of antioxidant therapy has been proposed to circumvent alcohol-induced apoptosis of neuronal cells, which underlies the decrease surface area. Specifically, vitamin C and E supplementation during pregnancy has been shown to reduce oxidative stress associated with alcohol exposure (for a review, see Cohen-Kerem et al., 2003). However, a recent study showed that treatment with mega-doses of anti-oxidants is associated with growth retardation
in alcohol-affected offspring (Goh et al., 2009). Therefore, further research is needed to
determine the safety and efficacy of such interventions.

Alternatively, behavioural interventions can be used to target the brain abnormalities of
ARND patients. Neuroimaging studies have shown that various training approaches can
induce structural anatomical changes in the brain, particularly in terms of increasing cortical
volumes. This phenomenon, known as neuroplasticity, has been observed in adults as well as
in the developing brain using various training approaches such as learning to play an
instrument (Hyde et al., 2009). Notably, brain changes were observed both in regions
relevant to the particular ability of interest as well as regions involved in heteromodal
integration functions. Such research suggests the possibility of using therapy interventions to
induce neuroplastic change in both in specific association areas such as the superior temporal
gyrus as well as more global areas of deficit.

In terms of global interventions, animal research suggests that postnatal environmental
enrichment can diminish the brain damage in ARND (Hannigan and Berman, 2000). For
example, compared to rats raised in isolation, those reared in large groups with sensory and
motor stimulation performed better on finding a submerged platform in the Morris water
maze task and had (Hannigan et al., 1991; Wainwright et al., 1993). This research offers
promise for facilitating recovery in ARND patients by providing them with more
opportunities interact with their peers and exposing them to a variety of sensory-motor
experiences in order to induce neuroplastic change in global domains.

More focused interventions can also be used to improve outcomes in specific areas of deficit.
For example, Klintsova et al. (2000) demonstrated that motor training in rats exposed
prenatally to alcohol resulted in an increase in the number of Purkinje cells in the cerebellum,
an area associated with motor functions. As such, training in social skills and language may help induce neuroplastic change in the right superior temporal region, which observed to be structurally abnormal in ARND. Similarly, repeated training on tasks involving visual attention to object shapes may ameliorate brain damage in the noted occipital-temporal region.

6.9 Conclusion

In summary, the current study showed that children with ARND have global reductions in total brain volumes and cortical volume. The cortical reductions did not reflect cortical thinning, but rather seem to reflect a reduction and sex dimorphism in global surface area. In particular, there was a significant group by sex interaction, with females in the ARND group showing greater cortical volume and surface area reductions than males. These reductions in global surface area may reflect a reduction of progenitor cells early in corticogenesis either due to apoptosis or a disruption of cell division. Beyond global effects, specific surface area abnormalities were also observed in the right temporal lobe, particularly in the occipital-temporal area as well as the superior temporal gyrus. Also observed was a global reduction in gyrification, which may be linked to alcohol’s effect on neuronal migration. Together, the reductions in global surface area and gyrification may explain the diffuse neurobehavioural deficits characteristic of ARND.
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