Effects of prenatal alcohol exposure on cerebellar volume in children: an MRI study

by

Natalie Rosella Boonzaier

(BNZNAT001)

SUBMITTED TO

THE UNIVERSITY OF CAPE TOWN

in fulfilment of the requirements for the degree

M.Sc (MED) (Applied Anatomy).

FACULTY OF HEALTH SCIENCES

September 2012

Supervisors: Professor Ernesta Meintjes and Dr. Christopher Warton

DECLARATION

I, ……, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

I empower the university to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature: ……

Date: ……

Abstract

BOONZAIER, N.R. M.Sc (Anatomy). Department of Human Biology, Faculty of Health Sciences, University of Cape Town, PO Observatory 7925, Cape Town, Republic of South Africa. *Effects of prenatal alcohol exposure on cerebellar volume in children: an MRI study.*

Years of research have demonstrated that prenatal exposure to alcohol contributes to a range of effects in exposed children. These include problems in attention and hyperactivity, deficits in memory and learning, and problems with social, as well as emotional development. Past research has demonstrated that the cerebellum is a significant target of the teratogenic effects of alcohol. The aim of this study was to determine whether prenatal exposure to alcohol has specific effects on the volumes of specific lobules of the cerebellum. Lobule tracing was performed manually, with Multitracer, using a refined methodology. Lobule volumes (normalized for total cerebellar cortical volume) were analysed as functions of diagnosis as well as alcohol exposure. Lobules IX and X were affected when analysing normalized volumes as a function of diagnosis, with the fetal alcohol syndrome diagnostic group being most specifically affected. Significant differences between sex groups were found only for right lobules I-V and left lobule VIII, and hemisphere differences were found in lobule X. When analysing normalized lobule volume as a function of alcohol exposure, in the left hemisphere, lobules I-V showed positive correlations with alcohol exposure, suggesting that this region is relatively spared. Lobule IX and the vermis of the right hemisphere showed negative correlations with alcohol exposure. The strongest negative correlations were found for measures of absolute alcohol per day averaged across the period of pregnancy as opposed to at time of conception. Overall findings suggest that prenatal alcohol exposure causes disproportionate reductions in volume in specific lobules of the cerebellums of children with fetal alcohol spectrum disorders.

Key words: cerebellum; lobular volumes; fetal alcohol spectrum disorders; manual tracing; high-resolution MRI

September, 2012

Acknowledgements

I offer my sincerest gratitude to my project supervisor, Associate Professor Ernesta Meintjes, for her patience and guidance during these past two years. I am forever grateful for the skills that I have learned under her guidance. I cannot overstate my gratitude to my co-supervisor, Dr. Christopher Warton, with whom I have worked for almost three years, during which I gained an immeasurable amount of knowledge. His wisdom and support will always be appreciated. I would like to thank the principal investigators, Professors Sandra and Joseph Jacobson, and Professor Chris Molteno for giving me the opportunity to work with them. I feel privileged to have been a part of this project.

I am grateful to Katya Mauff, from the Department of Statistics at The University of Cape Town, for her assistance with the statistical aspect of this Master's project; and I would like to thank my colleague, Steven Randall, for his statistical advice and his support. I would also like to thank Priya Lakshmi for generating the 3-D cerebellar images seen in this thesis.

There are various funders that, without whom, the overall project would not have been possible: NIH grants R01AA016781, R21AA017410, U01-AA014790; South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa; Medical Research Council of South Africa; Joseph Young Sr. Fund, State of Michigan; Ellison Medical Foundation; the University of Cape Town and Wayne State University. Additionally, I would like to thank the various funders that allowed me to pursue my Master's studies full-time: The Harry Crossley Foundation, The Ernst and Ethl Eriksen Trust, The Duncan Baxter Trust (UCT) and the NRF-DAAD/Grant-holder's funds.

I'd also like to thank the CUBIC radiographers Marie-Louise de Villiers and Nailah Maroof, Siemens South Africa, and our UCT and WSU research staff Nicolette Hamman, Mariska Pienaar, Maggie September, Emma Makin, Renee Sun and Neil Dodge.

Lastly, I thank my mother, Gina Boonzaier, and my brother, Nicolas, for supporting me throughout. I dedicate this to you.

TABLE OF CONTENTS

ABREVIATIONS

Abbreviation	
AA	absolute alcohol
ADHD	attention deficit and hyperactivity disorder
ARBD	alcohol-related birth defects
ARND	alcohol-related neurodevelopmental disorder
CCAS	cerebellar cognitive affective symdrome
CNS	central nervous system
CSF	cerebro-spinal fluid
DWS	Dandy Walker Syndrome
EPI	echo planar imaging
FAE	fetal alcohol effects
FAS	fetal alcohol syndrome
FASD	fetal alcohol spectrum disorder
FLOc	flocculus/lobule X (coronal plane)
FLOs	flocculus/lobule X (sagittal plane)
HE	heavily-exposed (non dysmorphic)
MPRAGE	multiecho magnetization prepared rapid gradient echo
MRI	magnetic resonance imaging
PET	positron emission tomography
pFAS	partial fetal alcohol syndrome
PUNc	pyramid, uvule and nodule (coronal plane)
PUNs	pyramid, uvule and nodule (sagittal plane)
SD	standard deviation
Shh	sonic hedgehog
TONc	tonsil/lobuleIX (coronal plane)
TONs	tonsil/lobuleIX (sagittal plane)

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 LITERATURE REVIEW

Prenatal Alcohol Exposure

Years of research have demonstrated that prenatal exposure to alcohol contributes to a range of effects in exposed infants. These include problems in attention and hyperactivity, deficits in memory and learning, and problems with social, as well as emotional development.

Although alcohol has been consumed for centuries, even by pregnant women, the detrimental effects of this substance only became a matter of interest during the 1970s, when Jones and Smith (1973) described the long-term consequences of prenatal exposure to alcohol. Animal models were developed to demonstrate its teratogenic effects. These effects included retardation of growth and physical abnormalities (Riley and McGee, 2005; Chernoff, 1978). Current research shows that prenatal alcohol exposure is responsible for a variety of adverse effects on the physical, neurological and behavioural development of the exposed fetus (Thomas *et al.*, 2010), and the first paper to describe these common dysmorphic and developmental problems found in fetal alcohol syndrome (FAS) was by the above-mentioned Jones and Smith, who eventually introduced the term "fetal alcohol syndrome". This term was used to describe a common pattern of deficits that were observed, which originally included pre- and postnatal growth deficiency, small head size with mental "subnormality", and facial abnormalities. It was after these descriptions that alcohol's teratogenicity became a great subject of interest, and the relationships between alcohol and fetal outcome began to be studied in depth.

Fetal Alcohol Syndrome

The diagnostic criteria characterizing FAS include: (1) specific distinctive craniofacial dysmorphology (short palpebral fissures, thin upper lip, flat or smooth philtrum) (Fig 1), (2) pre- and postnatal growth deficits, and (3) central nervous system (CNS) dysfunction (with a small head circumference and microcephaly as an indicator for CNS dysfunction) (Manning and Hoyme, 2007). Though all characteristics need to be present for a formal diagnosis of FAS, CNS dysfunction commonly occurs in fetal alcohol spectrum disorder (FASD) when the other FAS characteristics are not present (Fryer *et al.*, 2007). An additional criterion for diagnosis may include confirmed history of maternal alcohol abuse (Hoyme *et al.*, 2005).

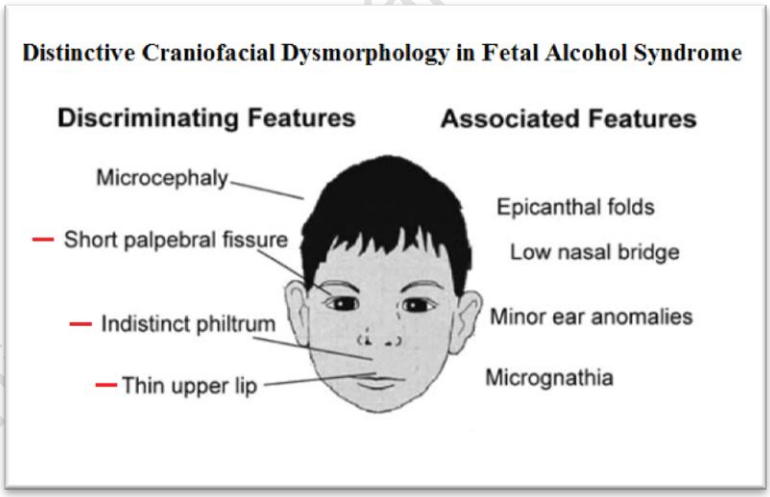


Figure 1: Typical facial phenotype found in children with FAS. The short palpebral fissure, thin upper lip and flat or smooth philtrum are features usually used for diagnosis, along with various other features that may be found. (Riley and McGee, 2005)

Fetal Alcohol Spectrum Disorders

FASD is a non-diagnostic umbrella term, which describes the entire range of effects that result from gestational exposure to alcohol (Spadoni *et al.,* 2007; Norman *et al.,* 2009), with FAS being the most severe of the FASDs (Jacobson and Jacobson, 2002). The other conditions that fall within the FASD category, but fall short of meeting all the diagnostic criteria of full-blown FAS, include partial FAS (pFAS), fetal alcohol effects (FAE), alcohol-related birth defects (ARBD), and alcohol-related neurodevelopmental disorders (ARND) (Hoyme *et al.,* 2005). FAE is generally diagnosed when only two of the three FAS criteria, as mentioned above, are met. This usually involves the subjects not meeting the craniofacial criteria (Autti-Rämö *et al.,* 2002). ARND involves the presence of cognitive difficulties such as deficits in attention, verbal difficulties or perceptual difficulties, which may be consistently exhibited without pre- or postnatal retardation of growth or the typical associated facies (Autti-Rämö *et al.,* 2002). Important parallels have been noted between FAS and ARND. Within the cognitive domain, attention, arithmetic and working memory are the features which are most severely affected consistently in both disorders; and within the behavioural domain, research has found increased impulsivity, social problems and aggression (Jacobson and Jacobson, 1999). The largest effect with regard to all conditions caused by prenatal alcohol exposure appears to be in the ADHD category. This association has been well-documented in various studies (Jacobson and Jacobson, 2002; Guerri *et al.*, 2009).

The Behavioural Phenotype Associated with Prenatal Alcohol Exposure

Determining the behavioural phenotypes that result from heavy prenatal alcohol exposure is important because the identification of these children is crucial for early interventions. Prenatal alcohol exposure has been reported to contribute to poorer maternal attachment and behavior-related problems in childhood (Kelly *et al.,* 2000), and data suggests that long-term deficits in adaptive and other behaviours may be present in individuals with prenatal alcohol exposure, even in the absence of cognitive deficits (Roebuck *et al.,* 1998). Though the behavioural short-comings may be caused by social and environmental factors (Jacobson and Jacobson, 2002), studies do suggest that prenatal exposure to alcohol may have effects on socio-

emotional development that are independent of the environment in which the child is raised (Brown *et al.,* 1991). Streissguth *et al.* (1991) reported in a study, looking at 61 adolescents and adults with FASD, that maladaptive behaviors such as distractibility, poor judgment and difficulty perceiving social cues were common, with the most prominent problems being the failure to consider the consequences of one's actions and poor inter-personal relationships. This study also stated that FAS is not a disorder limited to childhood; that there is long-term progression of the disorder into adulthood, where these maladaptive behaviors present the greatest challenge to management. Though the deficits mentioned are most severe and have been documented more extensively in children with FAS, children with other forms of FASDs (most probably exposed to lower levels of alcohol) often exhibit similar problems (Brown *et al.,* 1991; Kelly *et al.,* 2000; Jacobson and Jacobson 2002).

The societal burden of FASD is large in terms of suffering, loss of productivity, as well as excess medical and educational expenses (Hoyme *et al*., 2005). The economic cost associated with FAS-related growth retardation include: surgical repair of organic anomalies (including cleft palate, Tetralogy of Fallot) as well as treatment of sensorineural problems, and mental retardation (Abel and Sokol, 1987). Accurate estimates of the care costs for persons with FAS are necessary for the appropriate funding of health care, developmental disability services, specialised education, as well as prioritizing funding of public health prevention efforts (Lupton et al., 2004).

Effects of Prenatal Alcohol Exposure on Brain Development

Initial studies relied on postmortem autopsies, which demonstrated that severe prenatal alcohol exposure lead to microencephaly, which is a small brain, as well as neuroglial heterotopias, which is the migration of nerve cells to the wrong place. These studies also showed disrupted development of both the corpus callosum and cerebellum (Swayze *et al.,* 1997).

Postmortem autopsy cases were heavily relied on for the study of brain morphology in FAS prior to the implementation of neuroimaging techniques (Spadoni *et al.,*

2007). Even with this limitation, a wide range of neuropathological findings were observed. These findings included hydrancephaly (replacement of cerebral tissue with cerebral spinal fluid (CSF)), anencephaly (congenital absence of most of the cerebrum and spinal cord), holoprocencephaly **(**inability of the brain to divide into hemispheres or lobes causing insufficient development of facial characteristics), cerebral dysgenesis (defective cerebral development), abnormal neural migration, hydrocephaly (buildup of CSF in the ventricles), microcephaly (caused by microencephaly), enlarged/reduced/absent ventricles, and severe disorganization of the CNS. Further abnormalities were found in the basal ganglia, diencephalon, brain stem, optic nerve, olfactory bulb, hippocampus, pituitary, neural tube and the cerebellum (Spadoni *et al.,* 2007; Mattson *et al.,* 1992). Past structural imaging studies had also confirmed that prenatal alcohol exposure leads to size reductions in the anterior vermis of the cerebellum (O'Hare *et al.,* 2005; Steinlin, 2007; Goodlett *et al.,* 2005). Due to the unavailability of live imaging of the brain, the heterogenous brain malformations were concluded to have no pattern of association to FAS. Apart from these uncertainties, many postmortem cases described brain abnormalities (like those mentioned above) that were incompatible with life (Spadoni *et al.,* 2007). Current magnetic resonance imaging (MRI) studies are consistent with previous postmortem studies, reporting overall reductions in volumes of cranial, cerebral and cerebellar vaults in children with fetal alcohol spectrum disorders (FASD) (Mattson *et al.,* 1996; Archibald *et al.,* 2001; Sowell, 2002; Guerri *et al.,* 2009).

The development of MRI techniques has provided the opportunity to study the characteristics of the effects of prenatal alcohol exposure on the brains of live individuals, allowing study of a more generalized sample of the FASD population (Spadoni *et al.,* 2007). Structural anomalies can now be observed by brain region, and the behavioural correlates of these structural abnormalities can be studied. These studies have demonstrated specific alterations in the brains of individuals whom have been prenatally exposed to high doses of alcohol both with and without a diagnosis of FAS (Riley and McGee, 2005). Even when no obvious structural differences are apparent, a variety of imaging techniques such as MRI, and functional MRI (fMRI) have been used to demonstrate that prenatal exposure to alcohol also disrupts grey and white matter development (Norman *et al.,* 2009). An important finding of these

imaging techniques is that brain anomalies and dysfunction related to prenatal alcohol exposure can be present without exhibiting the characteristic facial dysmorphology, which is an important factor when identifying and diagnosing children with FASD (Hoyme *et al.*, 2005). Therefore, neuroimaging techniques have advanced the understanding of brain-behaviour relationships in FASD.

Various imaging studies have attempted to determine the effects of prenatal alcohol consumption on the CNS of the children affected. A voxel-based morphometry (VBM) study by Sowell *et al.* (2001) reported a set of results from a longitudinal study that looked at a sample of children and adolescents with maternal drinking histories. This longitudinal study reported significant group differences for total intracranial volume, total grey, total white matter volume and total CSF volume, with the exposed subjects having smaller volumes that controls in all cases. The cerebellum was not examined in the mentioned study, however, and the ages of the subjects ranged from 8-22 years, with 2 of the individuals being left-handed whereas the rest of the subjects were right-handed. Additionally, subjects with prenatal alcohol exposure were only diagnosed to either have FAS or prenatal exposure to alcohol (PEA), depending on facial dysmorphology. A study by Archibald *et al.* (2001) used manual tracing to analyse 14 participants diagnosed with FAS (mean 11.4 years), 12 participants with PEA (mean 14.8 years), and 41 age- matched control participants (mean 12.8 years). This study found a trend toward more severe cerebellar hypoplasia than cerebral hypoplasia. The cerebellum was studied as an entity and was not divided into subsections. Though all exposed subjects were reported to be born to alcoholic women, specific data regarding dose and timing of alcohol exposure was unknown. A later study by O'Hare *et al.* (2005) manually traced and analysed the cerebellums, using Multitracer (Woods, 2003), of the same sample of subjects that were also diagnosed as having either FAS or PEA, and with the same wide age range of 8-22 years. Alcohol-exposed individuals showed statistically significant reductions in all the cerebellar regions observed. However, the study only focused on regions of the midline vermis, which makes up only one subsection of the cerebellum.

Swayze *et al.* (1997) conducted a cross-sectional MRI study using retrospective information with a sample size of 10 individuals (4 children 3 adolescents and 3 adults). The study focused on brain anomalies as a result of prenatal alcohol exposure, with affected subjects falling under the diagnostic category of FAS only. 4 patients were found to have cerebellar dysgenesis. The study had very little statistical power as a result of the small sample size, with the subgroups spanning a large age range of between 8-26 years. By contrast, other studies have reported no significant results involving the cerebellum. For example, Clark *et al.* (2000) conducted a cross-sectional study on a sample of 19 individuals, with the age range of 16-30 years and diagnosis of FAS, and reported no statistically significant differences in the cerebellum. The study suggested that cerebellar damage may only be representative of individuals from the severe end of the continuum. In comparison to these reports, this study looked at a larger sample size with a narrow age range, with subjects diagnosed as either FAS, pFAS or heavily exposed (non dysmorphic), along with a control group. Additionally, this study analyzed a cohort from a prospective study with accurate maternal drinking histories, following the mothers since time of pregnancy. Furthermore, all cerebellar subsections were analyzed, not just the vermis.

It has been well-documented that severe prenatal alcohol exposure causes reductions in overall brain size, as well as reductions in overall size of specific structures, like the cerebellum (Riley and McGee, 2005; Sowell *et al.*, 2002; Archibald *et al.*, 2001). This can be dealt with by normalising the volumes in the statistics and reporting the measured areas as proportions or ratios. For example, Archibald *et al.* (2001) analysed the entire cerebellum as a proportion of the entire cranial vault (infratentoral plus supratentoral). Sowell *et al.* (2001) overcame this problem by measuring the total volumes of the structures of interest (total supratentorial intracranial volume, total grey matter volume, total white matter volume and total CSF volume), and assessed them for group differences. Multiple regression analysis was then used with predictors for group membership and total supratentorial intracranial volume to predict volume measures, and to determine how much of the group difference found in each tissue type was accounted for by generalised brain volume reduction.

Cerebellar Involvement in Fetal Alcohol Spectrum Disorders

Alcohol consumption during the third trimester of pregnancy is highly associated with damage to the cerebellum, hippocampus and prefrontal cortex, and the patterns of structural and functional anomalies vary depending on the coincidence of exposure with the critical periods of development (Riley and McGee, 2005).

Amongst the various brain structures that have been associated with FASD, the cerebellum has been identified to be particularly vulnerable to prenatal alcohol exposure (Guerri *et al.,* 2009) and, due to its involvement in the execution of posture, balance and coordination, the structural abnormalities, such as cerebellar cortical hypoplasia (Autti-Rämö *et al.,* 2002), observed may explain why individuals affected with FASD often display motor deficits including impaired balance as well as impaired bi-manual coordination (Roebuck *et al.,* 1998; Roebuck-Spencer and Mattson, 2004). In addition to motor behaviour, the cerebellum is also responsible for higher-order functions (Schmahmann, 2001b), such as cognitive and affective regulation (Baillieux *et al.,* 2008), emotion (Schmahmann, 2001a) and verbal cognition (O'Hare *et al.,* 2005). The cerebellum has also been shown to be involved in classical conditioning (Woodruff-Pak *et al.,* 2000) and, in conformity with this, impairments in eyeblink conditioning have been noted in children with FAS (Jacobson *et al.,* 2008). Two of the findings that are most commonly observed in individuals with prenatal alcohol exposure are reduced cerebellar surface area (Autti-Rämö *et al.,* 2002) and reduced cerebellar volume (Archibald *et al.,* 2001). Studies also show that, in addition to reduced cerebellar volume, localized damage to particular regions of the cerebellum, such as the vermis, may occur (O'Hare *et al.,* 2005). Autti-Ramo *et al.,* (2002) have demonstrated that deviation in vermis development is one of the most sensitive morphological indicators of the effects of prenatal exposure to alcohol. This was an observation found in every one of their 17 exposed subjects, including those that had been exposed during the first trimester only. Specific observations have included hypoplasia of the vermis and malformation of the posterior vermis, which appeared to be significantly correlated to poor results in Visual Attention tests. Another finding of this study was hypoplastic cerebellar hemispheres in subjects prenatally exposed to alcohol, with a significant correlation to poor results in the Repetition of Nonsense Words testing. In order to investigate the effects of prenatal alcohol exposure on cerebellar regions,

the subdivisions need to be individually analyzed along with the entire volume and size of the cerebellum.

Cerebellar Anatomy

The organization of the cerebellum and its integration within the central nervous system is related to its anatomical connections, which are architectonically specific (Voogd and Glickstein, 1998). Clinically, the different topographic regions of the cerebellum are associated with specific and distinctive syndromes (Makris *et al.*, 2003). Damage to the anterior vermis and paravermal regions result in the anterior-superior vermis syndrome, which is characterized by instability of posture and gait (Kieran, 2005). When the flocculonodular lobe is involved in cerebellar damage or malformation, balance and eye movements are affected. Hemespheric cerebellar damage and malformation has been associated with defective control of ipsilateral limb movement (Kieran, 2005). It has also been demonstrated that the cerebellum is involved in performing new functions that were previously not exercised. The importance of the cerebellum for carrying out new, unknown tasks is significant when considering that children, during development, have an increased need to learn new cognitive functions (Campitelli *et al.*, 2005).

Various inconsistencies exist with cerebellar nomenclature. A problem often encountered with the "vermis proper" is its absence in the anterior lobe. Anatomists approach this obstacle in various ways. Schmahmann and colleagues (1999) used the term "vermis" when referring to the midline structure, which can be clearly defined, in the posterior lobe, and they used the term "vermal area" when referring to the anterior lobe. Larsell and Jansen used the prefix "H" when referring to structures found beyond the "vermal areas". For this study, the structure visible in the inferior region of the posterior lobe was designated as the vermis. For the remainder of the cerebellum, a "medial" zone was designated.

Atlases used today include those by Schmahmann *et al.* (1999), as well as Duvernoy's Atlas of the Human Brain Stem and Cerebellum (Naidich *et al.*, 2009).

These atlases were developed using cerebellar label guidance from atlases and nomenclature systems that were developed by the likes of Larsell & Jansen (1972), Courchesne *et al.* (1989) and Press *et al.* (1989). Though the currently used atlas by Scmahmann and colleagues (1999) is based on the cerebellum of one individual, it serves as a template for accurate lobule identification, particularly because it uses the nomenclature as derived by Larsell and Jansen (1972).

In order to study the effects of prenatal alcohol exposure on the cerebellum, various parcellation and tracing methods need to be refined. This is due to the lack in consistency of white laminae distribution within the cerebellums of different individuals (whether healthy or not). This lack of consistency is more commonly observed within the inferior surface of the cerebellum at crus II, lobules VIIb and VIIIa.

Determining a Topographical Correlation with the Cerebellum and FASDs

The connections of the cerebellum with other brain regions, as well as the uniformity of the cortical architecture of the cerebellum and the specificity of the cerebrocerebellar pathways, gives the cerebellum the ability to control the great variety of behaviours that have been associated with it (Schmahmann, 2001b; Schmahmann, 2001a; Steinlin, 2007), and identifying specific pathways that are affected by prenatal alcohol exposure may help with early identification of behavioral aspects that may be affected during postnatal growth and development. Determining the exact effects of prenatal alcohol exposure on the cerebellum will enable the possibility of developing methods for identification of alcohol exposure. These methods are necessary because attainment of reliable alcohol exposure information is not always possible (Jacobson *et al.,* 2002), for example, when drinking is denied, when maternal memory is impaired, or when biological parents are not available (Thomas *et al.,* 2010). Ernhart *et al.* (1988) found that mothers, at 5 years postpartum, would retrospectively report higher drinking levels during pregnancy than they reported during their antenatal interviews – though this fact is relevant, neuroimaging is not feasible for diagnosis due to the high costs of MRI scanning.

Previous literature and current studies appear to be very limited with regard to information of the specific effects of prenatal alcohol exposure on the morphology of the cerebellum in children with FASDs. Information available mentions reductions in overall cerebellar size and volume, as well as displacement of and reduction in anterior vermis size and hypoplasia of the anterior lobe (lobules I-V). Subjects with FAS have also been shown to have a more severe displacement of the superior and anterior edges of the anterior vermis than individuals that have non-dysmorphic FASD. However, different studies do not report consistent results (Riley and McGee, 2005; O'Hare *et al.,* 2005). This study, therefore, aimed to look at the specific and detailed differences in cerebellar lobule volumes that occur in these children using manual tracing of high resolution magnetic resonance images.

Since ethanol is considered to be amongst the most common substances to impact the developing brain, and since prenatal alcohol exposure is one of the leading and most preventable causes of birth defects, mental retardation and neurodevelopmental disorders (Guerri *et al.,* 2009), more studies are needed to better understand what specifically happens in the brains of children affected with FAS. In addition to needing an improved understanding of this field, research is needed for developing systematic identification strategies as well as effective intervention strategies to improve the outcomes for individuals affected by FASDs (Spadoni *et al.,* 2007).

The aims of this study were to (1) determine whether specific structural and volumetric differences could be found in the cerebellar lobules amongst the subjects with FASD and the controls; and (2) if there were specific differences, in which FASD sub-group were these most significant, and to what extent do these differ amongst the various sub-groups.

CHAPTER 2

MATERIALS AND METHODS

2.1 PARTICIPANTS FOR SCANNING

Full details for participant recruitment and demographic information can be found in published work by Jacobson et al., (2008).

Participants included 75 right-handed male (n = 42) and female (n = 33) children that form part of a longitudinal cohort from the "Cape Coloured" community that were recruited prenatally to participate in the first prospective longitudinal study of children with FAS. Children were either diagnosed to have FAS (n = 9), pFAS (n = 18) or non-dysmorphic heavy exposure (n = 28) by expert dysmorphologists. An additional selection of subjects were included in this study as controls (n = 20). Median age at the time of scanning was 10.7 (10.37; 10.95) years.

Demographic Background and Maternal Alcohol Consumption

Mothers were recruited at an antenatal clinic, which served a Cape Town community that was selected for its high prevalence of heavy alcohol use (Croxford and Viljoen, 1999). Each mother was interviewed regarding her alcohol consumption. This was done using a timeline follow-back approach to determine the incidence and amount of drinking on a day-by-day basis during a typical 2-week period (Jacobson *et al.*, 2002). Volume was recorded for each type of beverage consumed each day, which was then converted to oz. absolute alcohol (AA) using multipliers proposed by Bowman, Stein, & Newton, (1975) (liquor—0.4, beer—0.04, wine—0.2). Six summary measures were constructed. These included average oz AA⁄ day at conception, AA⁄ day averaged across pregnancy, AA per drinking day (quantity per occasion) at conception and across pregnancy, and number of drinking days per week (frequency) at conception and across pregnancy. Any woman that reported a minimum of 14 drinks per week (1.0 oz AA/day) or at least 2 incidents of binge drinking (≥5 drinks per occasion) per month during the first trimester of pregnancy

was considered eligible and was invited to participate in the study. The next woman initiating antenatal care for whom gestation was within 2 weeks of the heavy drinking mother was also invited to participate, provided that she reported drinking < 7 drinks per week and did not binge drink.

MRI Data Acquisition

All subjects were scanned as part of on-going studies of FASD on the 3T Siemens (Siemens Medical Systems, Erlangen, Germany) Allegra MRI scanner at the Cape Universities Brain Imaging Centre (CUBIC). High-resolution T1 weighted images were acquired using a volumetric navigated (Tisdall *et al.*, 2012) multi echo magnetization prepared rapid gradient echo (MPRAGE) sequence (van der Kouwe *et al.*, 2008). Imaging parameters were: FOV: 256 x 256 mm; 128 sagittal slices, TR: 2530 ms; TE: 1.53/3.21/4.89/6.57 ms; TI: 1100 ms; Flip angle: 7°; voxel size: 1.3 x 1.0 x 1.3 mm^3. The 3D EPI navigator provided real-time motion tracking and correction (Tisdall *et al.*, 2012), which served to substantially reduce the presence of any motion artifacts in the structural imaging data.

MR images were manually traced using Multitracer (Woods, 2003) software on a tablet PC. Inter-rater reliabilities using independent measures of 10 cerebellums are to be determined by an expert neuroanatomist and interclass correlation. The following lobules of the cerebellum were traced and measured: Lobules I-V, VI, crus I, crus II, VIIb, VIIIa, VIIIb, IX and X. The vermis was also traced as an entity. Various lobules were traced in more than one plane- lobules VIIIa and b in sagittal as well as horizontal planes; and the vermis, lobule IX and X in sagittal and coronal planes.

2.2 TRACING

Tracing Protocol

The protocol used for tracing cerebellar lobules was an adaptation of the refined protocol developed by Dr. Christopher Warton from the Department of Human Biology at the University of Cape Town who co-supervised this project.

Measurements of cerebellum lobule volume were done using MultiTracer (Woods, 2003). File images were magnified to 4x magnification. Brightness was adjusted close to its maximum and contrast was generally set between 20 000 and 22 000 for best viewing results. Volumes were recorded using the 'trap volume' readings and exported to Microsoft Excel.

The midline slice was determined by identifying the sagittal slice in which the cerebral aqueduct was most clearly visible (generally, this was between slices 127-129). The exception to this was when the posterior margin of the vermis was not clearly visible. In this case, the first slice where the vermis was clearly visible was defined as the midline slice.

The lateral ends of the cerebellum were defined by the point where it disappeared when scrolling laterally through the sagittal slices. Other views (coronal and horizontal) were used for confirmation when these lateral 'tips' were unclear.

Each hemisphere was divided into medial, intermediate and lateral zones. This was accomplished by subtracting the slice numbers from the midline to the lateral tip and dividing them by 3. The vermis was not considered as a separate zone. This method of division was carried out for crus I, crus II and the cerebellum as a whole. Considering that the anterior lobe and tonsil are almost entirely confined to the medial zone; and while lobules VI and VIII virtually end at the junction of intermediate and lateral zones (i.e. are almost entirely confined to the medial and intermediate zones), these were divided as separate entities or with independent divisions – lobules I-V, lobule VIII and the tonsil as single entities; and lobule VI

into medial and lateral (no intermediate). Crus I and crus II, however, were prominent in all three zones and were divided into medial, intermediate and lateral.

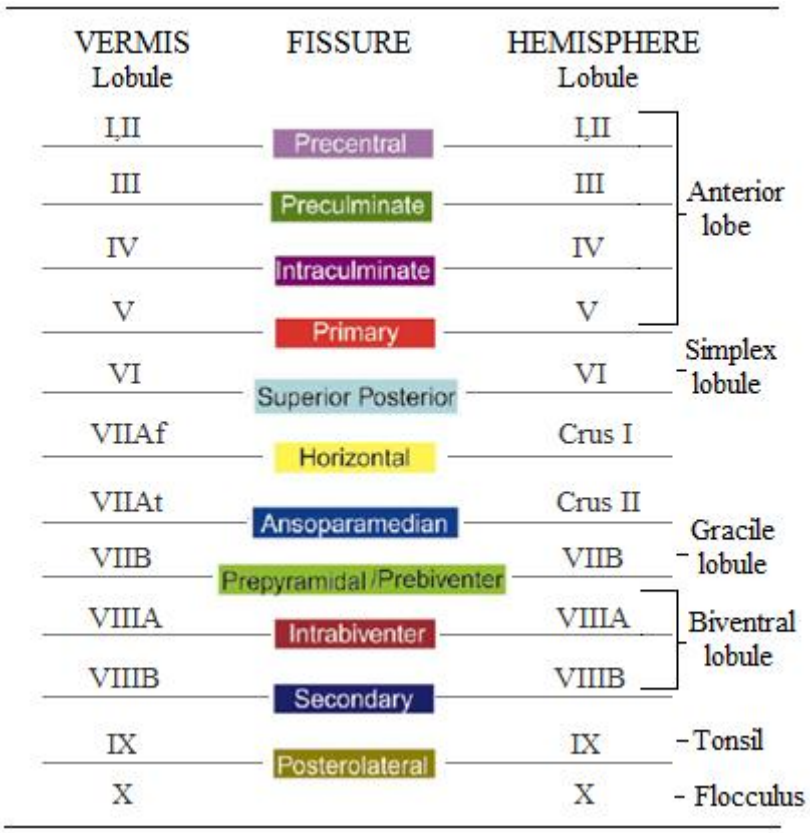
Volumetric measurements were obtained using an add-on method (i.e. The first volume obtained was that of the anterior lobe). With regard to lobules VI-X, the intermediate and lateral zone volumes were added to the relative medial zones subsequently.

Tracing was mainly performed sagittally. The coronal and horizontal views were used when necessary. The inferior cerebellum, which includes the pyramid, uvule and nodule were traced on the coronal plane, and it was these coronal measurements that were used for analysis of the inferior cerebellum.

The midline section as defined above was taken as the first slice for measurement on either side. Therefore, the slices from one cerebellum may have run from slice 77-128 on one side and 128-182 on the other.

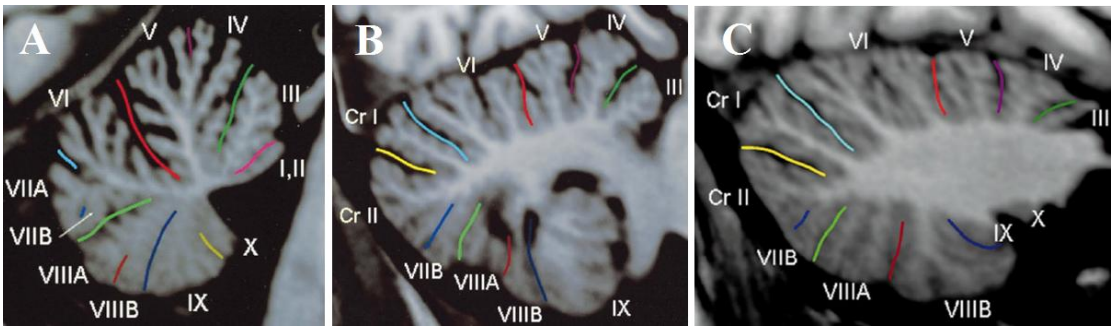
Lobules were defined and measured following guidelines set by Schmahmann *et al.,* (1999) (see Table 1 and Fig 2). Table 1, taken from the Three-Dimensional MRI Atlas developed by Schmahmann and colleagues, shows the lobule divisions that were used during lobule tracing in Multitracer. This colour scheme was used throughout when referring to images and lobule divisions. The decision to keep this revised nomenclature assisted in maintaining a reliable and simple methodology for fissure and lobule identification. The fissure names are anatomically consistent, and have historic significance. The above-mentioned authors decided to no longer use the Latin names of the lobules, and they used the Roman numeral designations as proposed by Larsell & Jansen (1972) instead.

Table 1: The relationships between fissures and lobules in the vermis and hemispheres (adapted from Schmahmann *et al.*, 1999)



Anterior lobe – Sagittal sections (lobules I-V)

Lobules IV and V of this lobe have strong resting state functional connections with the motor cortex (O'Reilly *et al.*, 2010). The primary fissure is the posterior boundary of this lobe. This could be easily defined in the midline slices but usually became more difficult to delineate as the images moved laterally. When difficulty was encountered after a few slices the scan was flipped to view the horizontal sections, where the fissure was drawn as a continuation of the one that was already defined, and this was used as a marker on the sagittal sections. The anterior lobe was measured as a single entity on each half of the cerebellums.



(taken from Schmahmann *et al.*,, 1999)

Figure 2: Sagittal MRI slices of the cerebellum showing the lobules using the specific colours that have been allocated to each fissure according to Schmahmann *et al.*, (1999), as seen in Table 1, beginning from the midline 'vermal area' (A) and moving out laterally (B and C).

Pyramid, uvule and nodule – Coronal sections (PUNc)

The vermis is a major target of the vestibular nuclei, and is a primary region for multimodal sensory and motor integration (Schmahmann, 2000). Coronal measurements were recorded as a single entity, and the markers left after this were used to trace the same entity in the sagittal view (PUNs) at a later stage of the tracing. This entity was not only measured next for guiding its tracing in the sagittal view, but also to guide the remainder of the lobules in the sagittal view.

Lobule IX – Coronal sections (tonsil - TONc)

Lobule IX has resting functional connectivity with the prefrontal cortex (O'Reilly *et al*., 2010). TONc was identified as the portion adjacent to the midline antero-inferiorly, which surrounds a specific "spray" or "hand" of laminae, which emerge from the medial surface of the centrum medullare (the mass of white matter in the centre of the hemisphere). This approach to measuring TONc was most reliable and the markers that resulted were used for guiding the sagittal tracing of this lobule (sagittal and coronal tracings should correspond). The tracing results of TONc were recorded as a single entity.

Lobule X – Coronal sections (flocculus – FLOc)

The flocculus, which has been reported to have somatosensory, autonomic and limbic connectivity (Roebuck *et al*. 1998), was initially visible near the pons, in line with the horizontal fissure and adjacent to the midline. Once identified, this lobule was followed and traced to the point where it could be joined with the tracing contours belonging to TONc.

Lobule VIIIa and VIIIb – horizontal sections(biventral lobule)

This lobule, which is a strong motor-correlated region (O'Reilly *et al*., 2010), was first measured in the horizontal plane, and the sagittal view, as mentioned above, was used to create initial markers. VIIIa was identified on the sagittal plane as the prominent lamina/e inferior to the centrum medullare and directly posterior to VIIIb, which was identified as the scoop-shaped lamina that curved anteriorly. After a few marks were made on the sagittal plane, the horizontal measurements of VIIIa were traced, followed by VIIIb.

Lobule VI – Sagittal sections (simplex lobule)

During motor tasks, lobule VI shows strongest resting state functional connectivity (O'Reilly *et al*., 2010), which extends to the above-mentioned lobules IV and V. Identification of crus I took place before tracing lobule VI. This was done using the coronal sections to create markers at the sagittal planes. Crus I was easily defined by the lamina of white matter, which it invariably contains. This lamina is prominent and runs posterolaterally with an upward convexity. This made following lobule VI laterally much easier. Lobule VI was divided into a medial and lateral portion.

Crus I – Sagittal sections

Crus I, reported to have some resting functional connectivity with the prefrontal cortex (O'Reilly *et al*., 2010), was found and traced between lobule VI and the horizontal fissure. It did not always exist at the midline but it rapidly grew and became apparent as the slices proceeded laterally**.** A small volume of the medial

cerebellum, known as the folium, which corresponds to crus I ('midline equivalent of crus I') was included with its medial measurements. Crus I extended prominently through all three zones (medial, intermediate and lateral).

Crus II – Sagittal sections

This lobule, which has also been reported to have resting functional connectivity with the prefrontal cortex (O'Reilly *et al*., 2010), needed to be traced with great attention. Its upper boundary, the horizontal fissure, was easy to define. The lower boundary, however, was problematic. The difficulty rested with identifying the ansoparamedian fissure, which separates crus II from VIIb. It was noted that the number of white matter laminae (and therefore fissures) was variable in this area. This is the stage at which the previous horizontal tracings of VIIIa horiz and VIIIb horiz were of assistance. The markers visible from these two lobules were used to identify lobule VIIb, which was the next prominent lamina, posterior to the markings of VIIIa horiz and VIIIb horiz. The fissure marking the posterior aspect of VIIb was identified as the ansoparamedian fissure. Once these boundaries were defined, crus II, which contains a prominent lamina that is invariably present, could be traced and divided into its medial, intermediate and lateral zones. The boundaries at the lateral zone were not always easy to define and needed to be done with care.

VIIb – Sagittal sections (gracile lobule - the remainder of lobule VII)

This lobule has been reported to have connections with the prefrontal cortex (O'Reilly *et al*., 2010) and, as mentioned above, was found immediately posterior to lobule VIII, which was defined on the horizontal plane. VIIb was not large but extended through all three zones.

Lobule VIII – Sagittal sections (biventral lobule)

VIIIa and VIIIb, which are motor correlated regions (O'Reilly *et al*., 2010), were not difficult to locate after their boundaries were defined in the horizontal views as stated before. VIIIa was identified on the sagittal plane as the prominent lamina/e inferior

to the centrum medullare and directly posterior to VIIIb, which was identified as the scoop-shaped lamina that curved anteriorly. VIIIa and VIIIb were generally easy to differentiate due to the presence of the intrabiventral fissure. The biventral lobule was not present at the midline as it was displaced by the tonsil (lobule IX). It is usually confined or almost entirely confined to the medial and intermediate zones. This lobule was measured as an entity and was not divided into its medial, intermediate and lateral zones.

Pyramid, uvule and nodule – Sagittal sections (PUNs)

Using the dots (markers) generated by the coronal drawings (PUNc), PUNs was traced for each hemisphere separately.

Lobule IX – Sagittal sections (tonsil – TONs)

This lobule was best observed in the coronal views and, therefore, tracing in the sagittal view was done by following the markers that were created by the coronal traces. These boundaries were difficult to define on the sagittal slices without the assistance of the coronal markers. Volumes from both the coronal and sagittal traces were recorded. Lobule IX was generally confined to the medial zone.

Lobule X – Sagittal sections (flocculus – FLOs)

The flocculus was very small and, when re-traced on the sagittal plane, was based on the markers created by the coronal traces. It was important to note that this structure may not have been very well delineated and measurements may not have been very accurate due to its small size.

White Matter – Sagittal sections

Since the cerebellar white matter was continuous with the large cerebellar peduncles, the definition of where one begins and the other ends was arbitrary. Therefore, the plane of the floor of the fourth ventricle was chosen as the cut-off point for the white

matter measurements. This plane was, therefore, based on a definite anatomical structure, which was always present and clearly visible. The plane was first drawn on horizontal slices and the markers generated were used to guide the final lines on the sagittal slices. The white matter was not separated into medial, intermediate and lateral zones, and was measured as a single entity.

2.3 SUMMARY OF THE TRACING PROTOCOL

In conclusion, the volumes recorded in the sagittal plane would be those of lobules I-V, lobule VI, Crus I, Crus II, lobule VIIb, lobules VIIIa and VIIIb, PUNs, lobule IX, lobule X and the white matter. Horizontal measurements for lobules VIIIa and VIIIb were also taken and, in the process of taking the sagittal and horizontal measurements, coronal volume measurements were generated for PUNc, lobule IX and lobule X.

Because the volumes were measured in an add-on manner, individual lobule volumes were calculated by subtraction on the spreadsheet, with the final file giving the total volume of the cerebellar hemisphere.

2.4 STATISTICAL ANALYSES

Statistical analyses were performed in Stata (Cohen, 1997). Although measurements were taken of various parts of the cerebellar lobules, including medial, intermediate and lateral segments of the respective lobules, often in multiple planes, only specific measurements of the lobules were analysed for this study. This included measurements of the "whole" lobules only. Measurements used were those of lobules I-V, lobule VI, crus I, crus II, lobule VIIb, lobule VIII, lobule IX, lobule X and the vermis. Furthermore, most measurements analysed were those that were taken from the sagittal sections of each lobule with the exception of the three coronal measurements that were taken for each hemisphere of each cerebellum (vermis, tonsil and flocculus). It must be noted that measurements were taken from left and right hemispheres independently. However, when running the relevant ANOVAs (see next paragraphs) there were no interactions between diagnosis and hemisphere

side. This did not necessarily mean that the hemisphere sides were equivalent, and it remained to be determined whether there were significant differences between the left and right hemispheres.

It has been well-documented that severe prenatal alcohol exposure has various effects on the nervous system. These may include abnormalities in brain shape, reductions in overall brain size, as well as reductions in overall size of specific structures, like the cerebellum (Riley and McGee, 2005; Sowell *et al.*, 2002; Archibald *et al.*, 2001). As a result, it was necessary to account for overall cerebellar cortical volume to determine whether the reductions in lobule volumes were over and above the reduction in overall cerebellar size. Therefore, our analyses used volumes after controlling for overall cerebellar cortical volume. Summary statistic means and standard deviation values were, therefore, reported as ratios or proportions as opposed to absolute volume measurements.

After controlling for total cerebellar cortical volume, the distribution of the normalized cortical volumes of lobules I-V, lobule VI, crus I, crus II, lobule VIIb, crusII + VIIb, lobule VIII, lobule IX, lobule X and the vermis were examined for normality. This was done to determine the future methodologies for statistical analyses.

Since normalized cortical volumes of the selected lobules were normally distributed, summary statistic tables were created of volume by diagnosis, volume by sex and volume by hemisphere (left/right), recording the means and standard deviations. The test statistic values were obtained using an ANOVA.

Box plots were created for each of the lobules of interest to examine differences between diagnostic groups. ANOVA and Bonferroni Pairwise Comparisons were performed to examine group differences.

Sex and hemisphere side were investigated as possible confounders of the relationship between normalized volumes and diagnosis. ANOVA's were used to examine volume differences due to sex and hemisphere side. Two-way ANOVA tests were run for diagnosis and volume concurrently with sex or hemisphere side to examine whether sex or hemisphere side were confounders of the effects of diagnosis on volume. This was done only in regions showing significant volume differences between diagnostic groups, with one within-subject factor (hemisphere) and one between-subject factor (diagnosis).

Bonferroni Test - After determining which lobule volume differences were significantly related to diagnosis, 6 tests were carried out amongst the diagnostic groups for the lobules of interest (lobules IX and X). This meant that, instead of comparing to the value $p = 0.05$, the reference p-value was adjusted to a smaller value ($0.05/6 = 0.008$). Using the Bonferroni adjusted comparative p-value, it was possible to identify between which diagnostic groups differences occurred for lobule IX and lobule X.

Correlations between Alcohol Exposure and Normalized Volume

Spearman Rank - Spearman Rank correlation tests were carried out to determine whether alcohol intake was associated with any differences in normalized cortical volume. The various measurements of alcohol exposure were not normally distributed and, therefore, this non-parametric test was used despite the fact that normalized volumes were normally distributed. The same test was carried out to determine the association of volume with age. After applying these tests, the correlation coefficient and the significance values were reported. After examining the correlations between alcohol exposure and normalised lobule volume, winsorisation (Hastings *et al.,* 1947) was carried out to correct for outliers.

CHAPTER 3

RESULTS

3.1 DEMOGRAPHIC INFORMATION

Demographic data, summarized in Table 3.1 below, displays the median and interquartile age ranges, as well as the median and interquartile range values for maternal alcohol intake over various time periods during pregnancy. All subjects were right-handed.

Table 3.1: Demographic information

Variable	FAS (n = 9)	pFAS (n = 18)	Heavy Exposed (n = 28)	Controls (n = 20)	Total (n = 75)	Chi-squared[#]
Male (n)	4	10	17	11	42	-
Age	10.4 (9.6; 10.8)	10.6 (10.2; 10.8)	10.9 (10.6; 11.6)	10.6 (10.3; 10.8)	10.7 (10.4; 10.9)	10.4 *
AA/day at conception	1.4 (1.3; 2.2)	1.2 (0.6; 2.0)	0.7 (0.2; 1.1)	0	0.5 (0; 1.3)	41.1 **
AA/drinking day at conception	4.6 (4.1; 7.6)	3.5 (2.3; 4.5)	2.5 (1.8; 3.6)	0	2.4 (0; 4.0)	40.7 **
Proportion drinking days per week at conception	0.2 (0.3; 0.4)	0.3 (0.3; 0.4)	0.2 (0; 0.3)	0	0.1 (0; 0.3)	36.9 **
AA/day averaged across pregnancy	0.8 (0.6; 1.7)	0.9 (0.4; 1.2)	0.3 (0.1; 0.8)	0	0.3 (0; 1.0)	44.7 **
AA/drinking day averaged across pregnancy	4.1 (4.1; 6.6)	3.4 (2.4; 4.6)	2.5 (1.9; 3.9)	0	2.3 (0; 4.0)	43.9 **
Proportion drinking days per week averaged across pregnancy	0.2 (0.2; 0.3)	0.3 (0.2; 0.4)	0.1 (0.1; 0.3)	0	0.1 (0; 0.3)	42.5 **
Total cerebellar cortical volume (mean ± SD), (mm^3)	49422 ± 3605	48943 ± 4957	53207 ± 5829	53778 ± 5165	-	-

Post-hoc = Man-Whitney: AA/day conception FAS>HE, Cntrl (p<0.05), pFAS>Cntrl (p<0.01), HE>Cntrl (p<0.001); AA/drinking day conception FAS>HE, Cntrl (p<0.03); All other variables of alcohol exposure FAS,pFAS,HE>Cntrl (p<0.001).

Median (interquartile range)

[#] Kruskal-Wallis chi-squared value

* $p < 0.05$

** $p < 0.0001$

Subjects with FAS and pFAS had higher values of prenatal alcohol exposure than the heavily exposed subjects. Although groups did differ in age, there were no significant relations of volume with age. Since the age ranges of the different diagnostic groups were appropriately narrow, it was not deemed necessary to control for age.

3.2 SUMMARIZED RESULTS

Group means and standard deviations (SD) of the lobule volumes (mm^3) before controlling for total cerebellar cortex are presented in Table 3.2.

Table 3.2: Cortical lobule volumes measured (mm^3)

Variable	FAS (n = 9)	pFAS (n = 18)	Heavy Exposed (n = 28)	Control (n = 20)	F-stat
Lobules I-V	5898 ± 1030	6238 ± 1275	6326 ± 1143	6472 ± 1114	1.06
Lobule VI	6626 ± 1683	6793 ± 1268	7211 ± 1233	7084 ± 1234.5	1.34
Crus I	12843 ± 2072	11845 ± 1835	13393 ± 2779	12966 ± 1911	3.35 *
Crus II	9470 ± 1673	9289 ± 2270	10567 ± 2127	10998 ± 2338	4.97 **
Lobule VIIb	3167 ± 1037	3140.5 ± 1031.3	3287 ± 870	3402 ± 1018	0.52
Crus II + VIIb	12638 ± 1727	12430 ± 2463	13855 ± 2573	14401 ± 2537	5.05 **
Lobule VIII	7395 ± 1403	7306 ± 1634	7526 ± 1295	7687 ± 1800	0.41
Lobule IX	1990 ± 379	2357 ± 546	2507 ± 589	2617 ± 494	6.32 ***
Lobule X	300 ± 85	377 ± 102	370 ± 114	375 ± 63	3.06 *
Vermis	2017 ± 379	2131 ± 372	2263 ± 379	2376 ± 424	4.5 **

Post-hoc = Tukey HSD: Crus I pFAS<HE ($p<0.01$); Crus II pFAS<HE, Cntrl ($p<0.03$, $p<0.005$, respectively); CrusII+VIIb pFAS<HE, Cntrl ($p<0.04$, $p<0.004$, respectively); Lob IX FAS<HE, Cntrl ($p<0.003$, $p<0.001$, respectively); Lob X FAS<pFAS,HE,Cntrl ($p<0.03$, $p<0.04$, $p<0.03$, respectively); Vermis FAS<Cntrl ($p<0.008$), pFAS<Cntrl ($p<0.04$).

Mean ± SD
* $p < 0.05$
** $p < 0.005$
*** $p < 0.0005$

Significant differences in volume were found as a function of diagnosis for crus I, crus II, crus II+VIIb, lobule IX, lobule X and the vermis.

3.3 DIFFERENCES IN NORMALISED VOLUME AS A FUNCTION OF DIAGNOSIS

After normalizing for total cortical volume of the cerebellum, summary statistic means and standard deviation values are reported as ratios or proportions, as opposed to absolute volume measurements. Normalized lobule volumes were all normally distributed. Tables 3.3a and 3.3b show results when analysing left and right hemispheres independently, and Table 3.3c shows results for left and right hemispheres together.

Table 3.3a: Summary statistics for normalized lobule volume by diagnosis (left)

Variable	FAS (n = 9)	pFAS (n = 18)	Heavy Exposed (n = 28)	Control (n = 20)	F-stat
Lobules I-V	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.48
Lobule VI	0.14 ± 0.03	0.14 ± 0.02	0.14 ± 0.03	0.13 ± 0.02	0.5
Crus I	0.27 ± 0.03	0.25 ± 0.05	0.25 ± 0.04	0.25 ± 0.03	0.50
Crus II	0.19 ± 0.04	0.19 ± 0.04	0.2 ± 0.03	0.2 ± 0.04	0.22
Lobule VIIb	0.07 ± 0.02	0.06 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.05
Crus II+VIIb	0.25 ± 0.03	0.26 ± 0.05	0.26 ± 0.04	0.27 ± 0.04	0.29
Lob VIII	0.14 ± 0.03	0.14 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	0.19
Lob IX	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.047 ± 0.009	1.32
Lob X	0.006 ± 0.002	0.008 ± 0.003	0.007 ± 0.002	0.007 ± 0.001	1.83
Vermis	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.70

Mean ± SD

Table 3.3b: Summary statistics for normalized lobule volume by diagnosis (right)

Variable	FAS (n = 9)	pFAS (n = 18)	Heavy Exposed (n = 28)	Control (n = 20)	F-stat
Lobules I-V	0.12 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.19
Lobule VI	0.13 ± 0.03	0.14 ± 0.02	0.13 ± 0.02	0.13 ± 0.02	0.34
Crus I	0.25 ± 0.04	0.24 ± 0.04	0.25 ± 0.03	0.23 ± 0.03	0.98
Crus II	0.2 ± 0.04	0.19 ± 0.04	0.2 ± 0.03	0.21 ± 0.03	1.46
Lobule VIIb	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.56
Crus II+VIIb	0.26 ± 0.04	0.25 ± 0.03	0.26 ± 0.04	0.27 ± 0.03	0.93
Lob VIII	0.16 ± 0.02	0.15 ± 0.03	0.14 ± 0.02	0.14 ± 0.03	1.13
Lob IX	0.041 ± 0.008	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.008	1.80
Lob X	0.006 ± 0.001	0.007 ± 0.001	0.007 ± 0.002	0.007 ± 0.001	1.87
Vermis	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.63

Mean ± SD

Table 3.3c: Summary statistics for normalized lobule volume by diagnosis (left and right)

Variable	FAS (n = 9)	pFAS (n = 18)	Heavy Exposed (n = 28)	Control (n = 20)	F-stat
Lobules I-V	0.12 ± 0.02	0.13 ± 0.02	0.12 ± 0.02	0.12 ± 0.02	0.62
Lobule VI	0.13 ± 0.03	0.14 ± 0.02	0.14 ± 0.02	0.13 ± 0.02	0.34
Crus I	0.26 ± 0.04	0.24 ± 0.05	0.25 ± 0.03	0.24 ± 0.03	1.34
Crus II	0.19 ± 0.04	0.19 ± 0.04	0.19 ± 0.03	0.20 ± 0.04	1.28
Lobule VIIb	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.02	0.19
Crus II+VIIb	0.26 ± 0.03	0.25 ± 0.04	0.26 ± 0.04	0.27 ± 0.04	1.02
Lob VIII	0.15 ± 0.03	0.15 ± 0.03	0.14 ± 0.02	0.14 ± 0.03	0.88
Lob IX	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	3.00 *
Lob X	0.006 ± 0.002	0.008 ± 0.002	0.007 ± 0.002	0.007 ± 0.001	3.56 *
Vermis	0.041 ± 0.006	0.043 ± 0.006	0.043 ± 0.007	0.004 ± 0.008	1.34

Mean ± SD

* $p < 0.05$

When analysing the left and right hemispheres separately, no significant differences were found for any of the normalized lobule volumes (Tables 3.3a and 3.3b). When analysing lobules of the left and right hemispheres together, however, significant differences in normalized lobule volumes were found only in lobule IX and lobule X (F = 3.0, p = 0.03 and F = 3.56, p = 0.01, respectively). These results were investigated further using pairwise comparisons to determine amongst which diagnostic groups these differences were significant.

Pairwise Comparison Tests were run for lobules IX and X in order to determine which diagnostic groups were associated with the normalized volume differences found in the above-mentioned lobules. Figures 3 and 4 show 3-D representations, generated in ITK-SNAP (Yushkevich *et al.,* 2006), and contours in Multitracer for lobules IX and X, respectively. Figures 5 and 6 show box plots of volumes for lobules IX and X, respectively, as a function of diagnostic group.

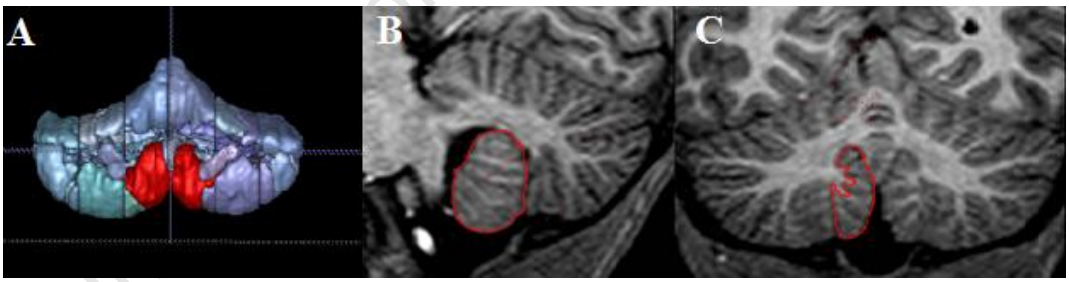


Figure 3: Images of lobule IX. A 3-D representation of the anterior view of a cerebellum with lobule IX for the left and right hemispheres in red (A), as well as the sagittal (B) and coronal (C) views of the lobule IX traces.

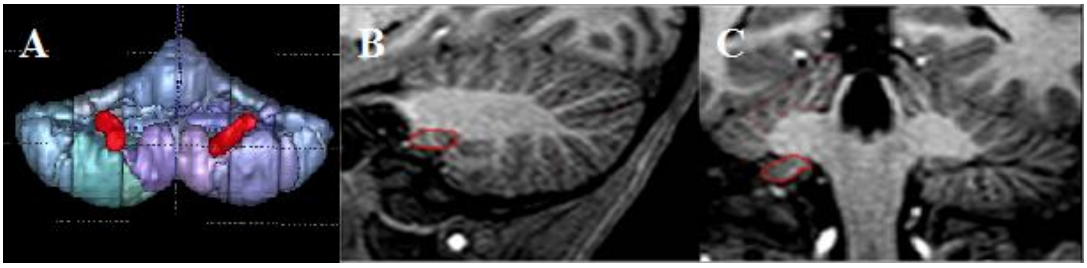
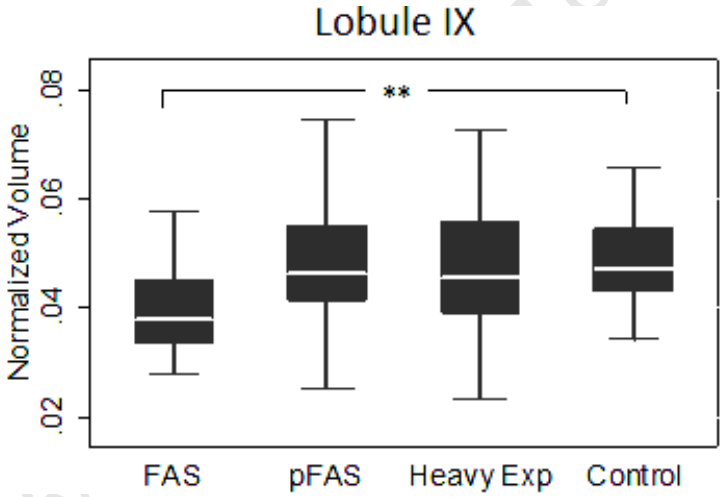


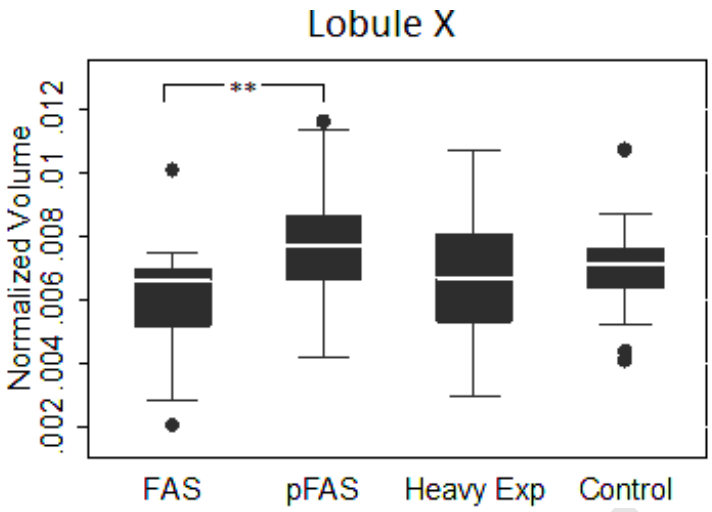
Figure 4: Images of lobule X. A 3-D representation of the anterior view of a cerebellum with lobule X for the left and right hemispheres in red (A), as well as the sagittal (B) and coronal (C) views of the lobule X traces.



** $p < 0.008$, p (Bonferroni) <0.05

Figure 5: Box plot of normalized volume for lobule IX as a function of diagnostic group.

In Figure 5, median values of normalized volume in lobule IX were lower in FAS than in the other diagnostic groups, particularly, the Control group. Pairwise comparisons demonstrated that significant differences were found only between the FAS group and the control group (T = -3.42, p = 0.001).



** $p < 0.008$, p (Bonferroni) < 0.05

Figure 6: Box plot of lobule X normalized volume as a function of diagnostic group.

In figure 6, the FAS group had the lowest median value for normalized volume in lobule X. Pairwise comparisons demonstrated that differences were significant only between the FAS and the pFAS groups ($T = -2.87$, $p = 0.005$).

3.3.1 Sex and Hemisphere

Differences associated with sex and hemisphere were independent of those associated with diagnosis.

- **Sex**

Tables 3.4a and 3.4b show mean and standard deviation values of normalized lobule volumes for males and females, for left and right hemispheres, respectively.

Table 3.4a: Summary statistics for normalized volume by sex (left)

Variable	Male (n = 42)	Female (n = 33)	F-stat of Sex
Lobs I-V	0.12 ± 0.02	0.11 ± 0.02	1.45
Lob VI	0.13 ± 0.02	0.14 ± 0.02	2.15
Crus I	0.22 ± 0.03	0.25 ± 0.04	0.01
Crus II	0.19 ± 0.03	0.20 ± 0.04	1.33
Lob VIIb	0.06 ± 0.01	0.06 ± 0.01	0.88
Crus II + VIIb	0.25 ± 0.03	0.26 ± 0.04	0.45
Lob VIII	0.14 ± 0.02	0.13 ± 0.02	4.64 *
Lob IX	0.04 ± 0.01	0.04 ± 0.01	1.35
Lob X	0.007 ± 0.001	0.007 ± 0.002	0.54
Vermis	0.04 ± 0.007	0.04 ± 0.006	1.78

Mean ± SD
* $p < 0.05$

Table 3.4b: Summary statistics for normalized volume by sex (right)

Variable	Male (n = 42)	Female (n = 33)	F-stat of Sex
Lobs I-V	0.12 ± 0.01	0.11 ± 0.01	4.74 *
Lob VI	0.12 ± 0.01	0.13 ± 0.02	3.53 †
Crus I	0.24 ± 0.03	0.24 ± 0.03	0.16
Crus II	0.19 ± 0.03	0.20 ± 0.03	1.43
Lob VIIb	0.06 ± 0.01	0.05 ± 0.01	0.28
Crus II + VIIb	0.25 ± 0.03	0.26 ± 0.03	0.93
Lob VIII	0.15 ± 0.02	0.14 ± 0.02	1.81
Lob IX	0.04 ± 0.01	0.04 ± 0.01	1.44
Lob X	0.006 ± 0.001	0.006 ± 0.001	0.06
Vermis	0.04 ± 0.006	0.04 ± 0.006	0.56

Mean ± SD
† $p < 0.1$
* $p < 0.05$

Significant differences with sex are seen in right lobules I-V and left lobule VIII. In both these regions, normalized lobule volumes are smaller in females than males. In right lobule VI, normalized lobule volume tends to be bigger in females.

Figures 7 and 8 show 3-D representations, generated in ITK-SNAP, and contours in Multitracer for lobules I-V and VIII, respectively. Figures 9 and 10 present plots for lobules I-V and lobule VIII, respectively, of normalized volume against sex.

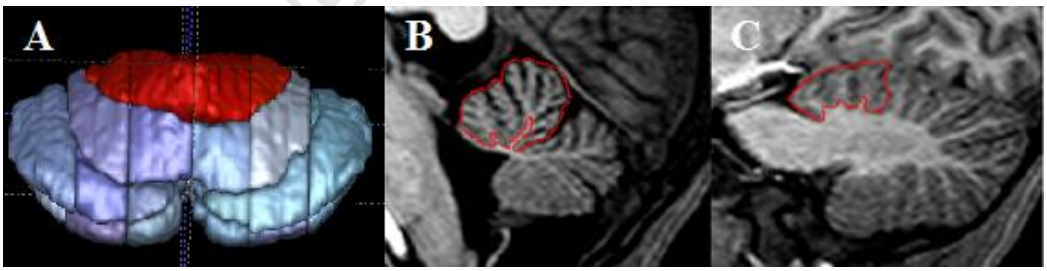


Figure 7: Images of lobules I-V. A 3-D representation of the posterior view of a cerebellum with lobules I-V from the left and right hemispheres in red (A), as well as the sagittal (B) and coronal (C) views of the traces of lobules I-V.

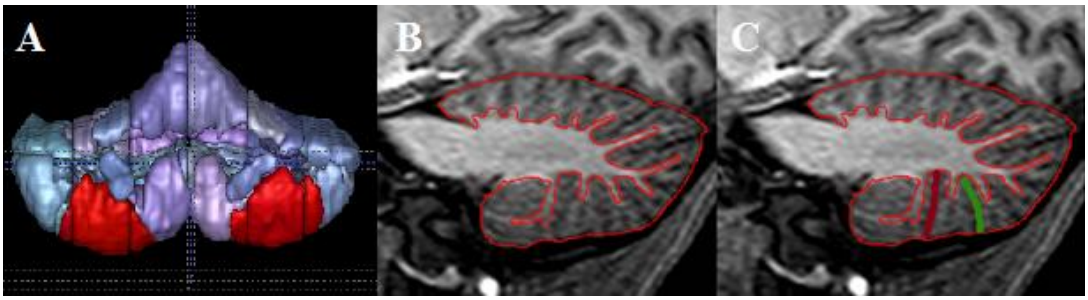


Figure 8: Images of lobule VIII. A 3-D representation of the anterior view of a cerebellum with lobule VIII from the left and right hemispheres in red (A), as well as the sagittal view, which shows contour data of the lobule VIII trace added to the trace of lobules I-VII (which is how the lobules were traced as described in the methodology (B). The fissures as described by Schmahmann *et al.*, (1999) can be seen in the additional sagittal section, with the thick maroon line representing the intrabiventer fissure, which separates VIIIa from VIIIb, and the green line representing the prepyramidal/prebiventer fissure, separating lobule VIII from VIIb (C).

* $p < 0.05$

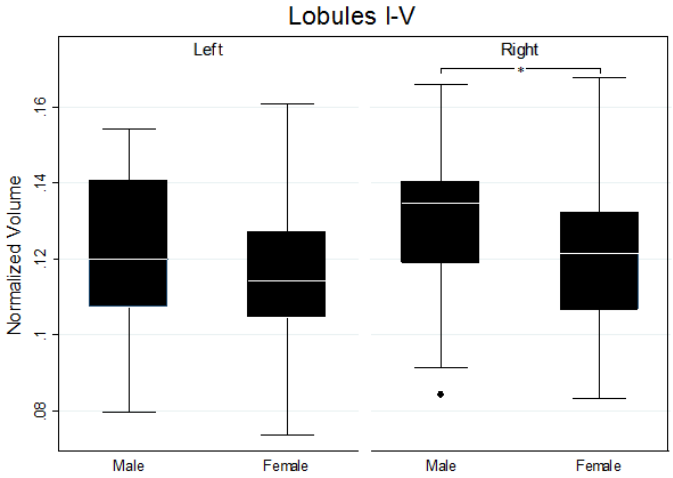
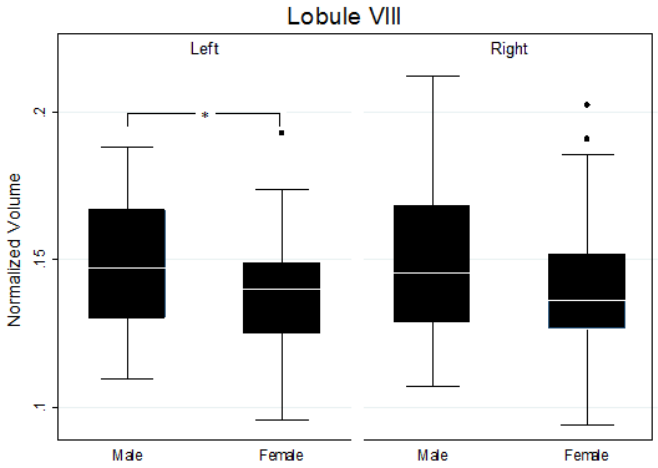


Figure 9: Box plot of normalized volume of lobule I-V as a function of sex.



***** $p < 0.05$

Figure 10: Box plots of normalized volumes of lobule VIII as a function of sex.

A two-way ANOVA looking at normalized lobule volume differences and sex, concurrently with diagnosis, was used to determine whether relationships between normalized volumes in lobules IX and X and diagnosis were affected when accounting for sex. Significant differences remained (F = 3.33, p = 0.02 and F = 3.52, p = 0.02, respectively) for lobules IX and X, respectively, for normalized volume and diagnosis**.** Further, there was no interaction between sex and diagnosis. Hence, sex is not a confounder.

- **Hemisphere Side**

Table 3.5 shows mean and standard deviation of lobular volumes for the left and right hemispheres. Only in lobule X is there a significant difference between left and right volumes (Figure 11). Normalized right hemisphere volumes are smaller than left hemisphere volumes in this lobule. Two-way ANOVA's were run for diagnosis and hemisphere side concurrently to determine whether significant volume differences remained for lobules IX and X after accounting for hemisphere side. Significance for normalized volume in lobule X and diagnosis remained when running tests concurrently (F = 3.66, p = 0.01). Effects of hemisphere did not interact with results that tested normalized volume and diagnosis. As such, hemisphere side is not a confounding factor.

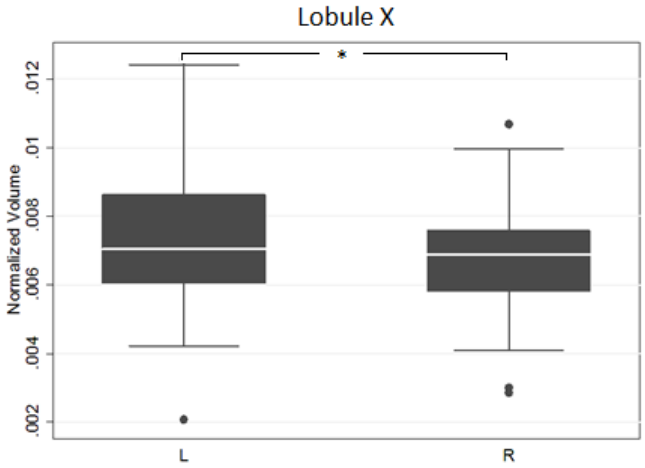
Table 3.5: Summary statistics for normalized lobule volume by hemisphere

Variable	Left	Right	F-stat for Hemisphere
Lobs I-V	0.12 ± 0.02	0.12 ± 0.02	3.34 †
Lob VI	0.14 ± 0.02	0.13 ± 0.02	0.69
Crus I	0.25 ± 0.04	0.24 ± 0.03	3.10 †
Crus II	0.19 ± 0.04	0.19 ± 0.03	0.49
Lob VIIb	0.07 ± 0.02	0.06 ± 0.01	3.72 †
Crus II + VIIb	0.26 ± 0.04	0.26 ± 0.03	0.04
Lob VIII	0.14 ± 0.02	0.15 ± 0.03	0.97
Lob IX	0.05 ± 0.01	0.05 ± 0.01	0.27
Lob X	0.007 ± 0.002	0.007 ± 0.001	4.79 *
Vermis	0.04 ± 0.007	0.04 ± 0.007	0.01

* Mean ± SD

† $p < 0.1$

* $p < 0.05$



* $p < 0.05$

Figure 11: Box plots of normalized volumes of lobule X as a function of hemisphere side.

3.4 DIFFERENCES IN NORMALISED VOLUME AS A FUNCTION OF ALCOHOL EXPOSURE

Spearman Rank correlation tests were carried out to determine whether maternal alcohol intake throughout pregnancy was associated with any differences in normalized cerebellar cortical volume that were seen amongst the exposed groups. The various measurements of alcohol exposure were not normally distributed and, therefore, non-parametric tests were used despite the fact that volumes were normally distributed. Left and right normalized lobule volumes were tested separately.

Left Hemisphere

Table 3.6 represents the correlations for the left hemisphere, followed by figure 12, which graphically represents the correlations in the form of a scatter plot for lobules I-V. Proportion of drinking days per week at time of conception is weakly associated with greater normalized volume in lobules I-V.

Table 3.6: Correlations of normalized volume measurements with different measures of alcohol intake (Left)

Variable		Lobule									
		Lobs I-V	Lob VI	Crus I	Crus II	Lob VIIb	Crus II+VIIb	Lob VIII	Lob IX	Lob X	Vermis
AA/day at time of conception	Correlation Coefficient	0.18	-0.08	0.12	-0.07	0.02	-0.04	-0.06	-0.20†	-0.05	-0.07
AA/drinking day at time of conception	Correlation Coefficient	0.10	-0.002	0.12	-0.11	0.04	-0.08	-0.06	-0.18	-0.02	-0.04
Proportion of drinking days/week at time of conception	Correlation Coefficient	0.23*	-0.13	0.12	-0.05	-0.006	-0.04	-0.09	-0.16	-0.05	-0.11
AA/day across pregnancy	Correlation Coefficient	0.17	-0.06	0.10	-0.12	0.07	-0.08	0.02	-0.19	-0.02	-0.11
AA/drinking day across pregnancy	Correlation Coefficient	0.05	0.04	0.05	-0.06	0.02	-0.05	-0.01	-0.18	-0.05	-0.03
Proportion of drinking days/week across pregnancy	Correlation Coefficient	0.20†	-0.10	0.11	-0.10	0.06	-0.08	0.01	-0.15	-0.02	-0.15

AA = Absolute Alcohol

† $p < 0.1$

* $p < 0.05$

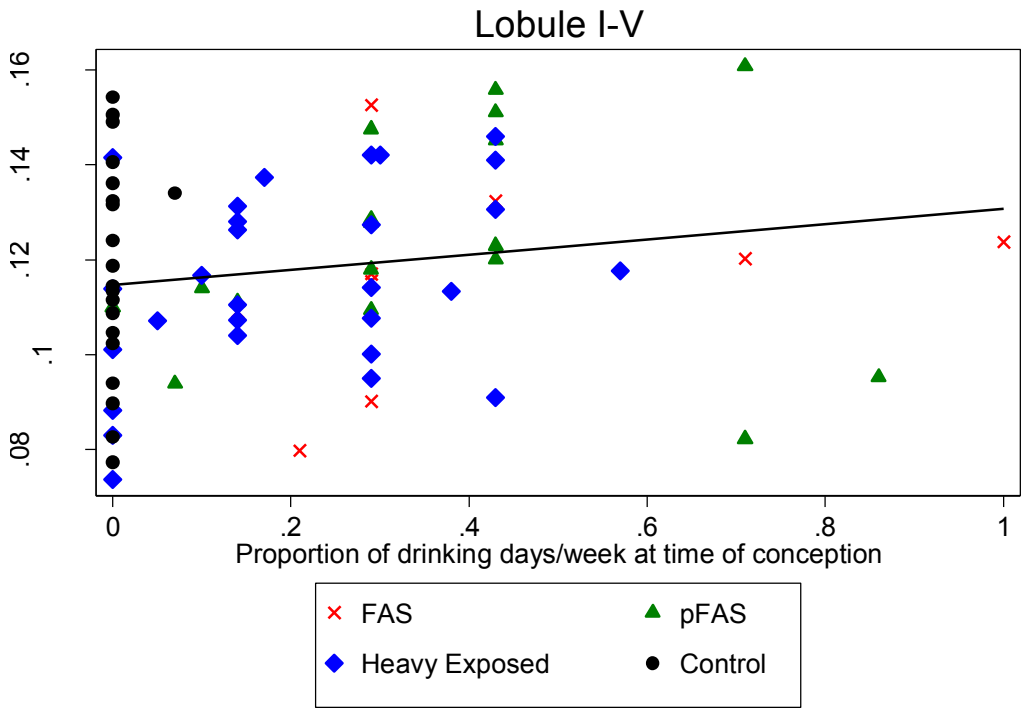


Figure 12: Plot of normalized volume of lobules I-V of the left hemisphere as a function of proportion of drinking days/week at time of conception.

Right Hemisphere

Tables 3.7 and 3.8 represent the correlations for the right hemisphere, before and after winsorisation of outliers, respectively. Significant correlations were found between all alcohol exposure variables and normalized volume for lobule IX; and a significant (of the 10th percentile) correlation was found with the vermis and proportion of drinking days per week across pregnancy. In all instances, increased alcohol exposure was associated with smaller volumes for lobule IX and the vermis. Outliers were winsorised for "AA/day across pregnancy" and "proportion drinking days/week across pregnancy" since these variables showed the strongest associations with volume. Winsorisation removes the extreme values of the data set and reduces the effects that the outliers may have exaggerated.

Table 3.7: Correlations of normalized volume measurements with different measures of alcohol intake (Right)

Variable		Lobs I-V	Lob VI	Crus I	Crus II	Lob VIIb	Crus II+VIIb	Lob VIII	Lob IX	Lob X	Vermis
AA/day at time of conception	Correlation Coefficient	0.03	-0.02	0.14	-0.06	0.02	-0.06	0.14	-0.31**	-0.01	-0.12
AA/drinking day at time of conception	Correlation Coefficient	0.03	-0.01	0.12	-0.10	0.04	-0.09	0.14	-0.29**	-0.02	-0.07
Proportion of drinking days/week at time of conception	Correlation Coefficient	0.06	-0.03	0.14	-0.01	0.01	-0.03	0.06	-0.27*	0.01	-0.15
AA/day across pregnancy	Correlation Coefficient	0.07	-0.01	0.11	-0.11	0.03	-0.11	0.18	-0.34***	-0.06	-0.15
AA/drinking day across pregnancy	Correlation Coefficient	-0.03	-0.01	0.09	-0.06	0.02	-0.05	0.14	-0.29**	-0.03	-0.04
Proportion of drinking days/week across pregnancy	Correlation Coefficient	0.08	-0.02	0.11	-0.08	0.03	-0.08	0.13	-0.32***	-0.06	-0.20†

AA = Absolute Alcohol

† $p < 0.1$

* $p < 0.05$

** $p < 0.01$

*** $p < 0.005$

Table 3.8: Correlations of normalized volume measurements with different measures of alcohol intake after winsorization (Right)

		Lobule									
Variable		**Lobs I-V**	**Lob VI**	**Crus I**	**Crus II**	**Lob VIIb**	**Crus II+VIIb**	**Lob VIII**	**Lob IX**	**Lob X**	**Vermis**
AA/day across pregancy	Correlation Coefficient	0.07	-0.01	0.11	-0.11	0.03	-0.11	0.18	-0.34***	-0.06	-0.15
Proportion of drinking days/week across pregnancy	Correlation Coefficient	0.08	-0.02	0.11	-0.08	0.03	-0.08	0.13	-0.32***	-0.06	-0.19†

AA = Absolute Alcohol

† $p < 0.1$

*** $p < 0.0001$

Figure 13 shows the negative association between volume of right lobule IX and absolute alcohol per day averaged across pregnancy before and after winsorisation. Figure 14 shows a 3-D representation, generated in ITK-SNAP, and contours in Multitracer for the vermis. Figure 15 represents the weak negative association of the volume of the right vermis with proportion drinking days per week averaged across pregnancy, before and after winsorisation.

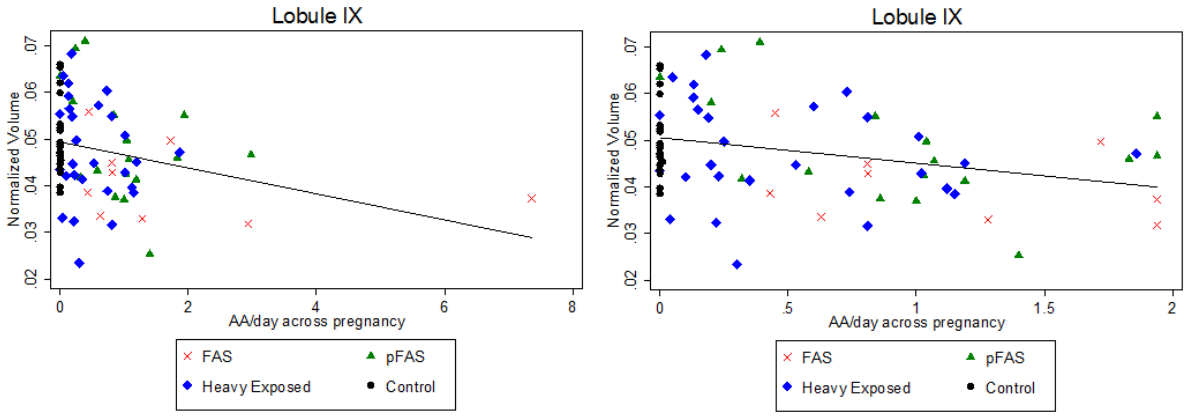


Figure 13: Negative correlation of normalised volume of right lobule IX with absolute alcohol per day averaged across pregnancy, before (left) and after (right) winsorization.

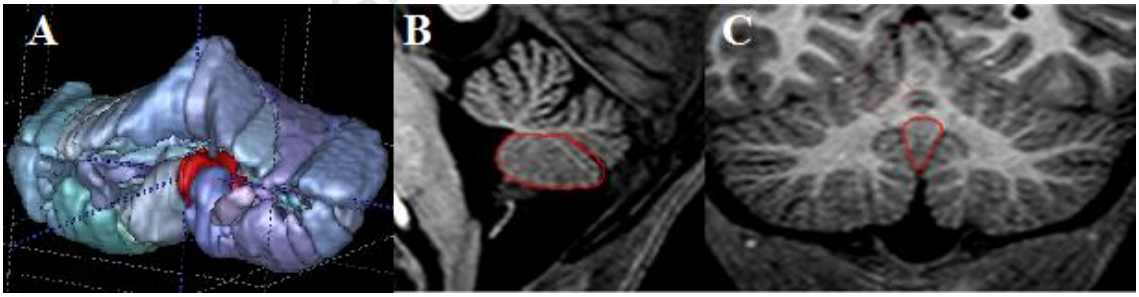


Figure 14: Images of the vermis. A 3-D representation of the antero-lateral view of a cerebellum with the vermis in red (A), as well as the mid-sagittal view (B) and a coronal view of the vermis traces (C).

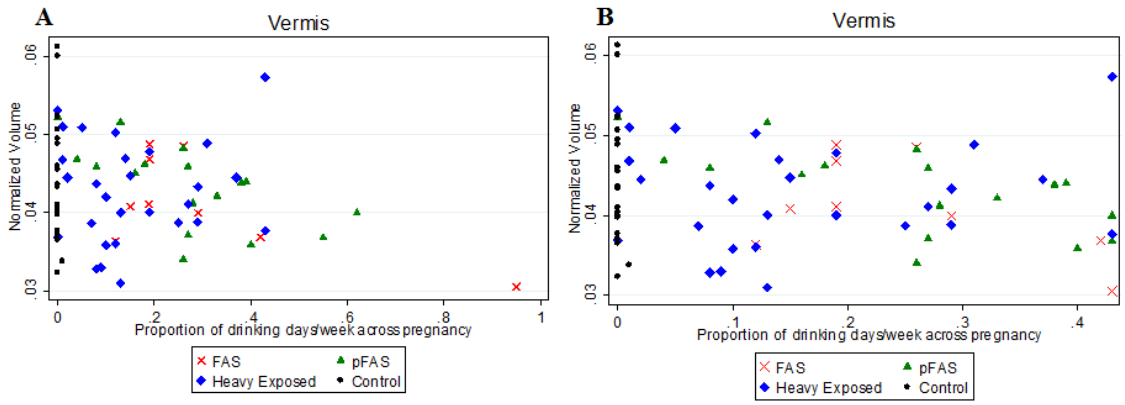


Figure 15: Negative correlation of normalised volume of the right vermis with proportion of drinking days per week averaged across pregnancy, before (left) and after (right) winsorization.

The associations of normalized volume in lobules IX and vermis with alcohol exposure remain significant after winsorisation.

CHAPTER 4

DISCUSSION

Overall findings of this study suggest that prenatal exposure to alcohol results in smaller volumes in specific lobules of the cerebellum in children with FASDs. Normalized lobule volumes were analysed as functions of diagnostic group and alcohol exposure independently and, in both instances, significant findings were observed. Lobules IX and X were affected when analysing normalized volumes as a function of diagnosis, with the FAS diagnostic group being most specifically affected. Significant differences between sex groups were found only for right lobules I-V and left lobule VIII, and hemisphere differences were found in lobule X. When analysing normalized lobule volume as a function of alcohol exposure, in the left hemisphere, lobules I-V showed positive correlations with alcohol exposure, suggesting that this region is relatively spared. Lobule IX and the vermis of the right hemisphere showed negative correlations with alcohol exposure. The strongest negative correlations were found for measures of absolute alcohol per day averaged across the period of pregnancy as opposed to at time of conception.

This discussion will focus on the main findings of this study and will suggest possible explanations for the results that were obtained, as well as suggestions for future research of this nature. Suggestions are proposed regarding what influences alcohol may have on the development of the cerebellums of the subjects. This data could advance our understanding of which functional domains are associated with particular parts of the cerebellum by comparing these results with findings from behavioural studies of FASD.

4.1 MAIN FINDINGS

Volume differences in Lobule X

The mean normalized volume of lobule X was smaller in the FAS diagnostic group, compared to all the diagnostic groups, but was only significantly smaller (T = -2.87) than the pFAS group. The flocculonodular lobe has been reported to be involved in facilitating gaze during linear head movements and for maintaining postural stability and orientation relative to gravity (Anand *et al.,* 1959). The study that reported this also suggested the important involvement of this lobe in processing signals that arise from the sacculi, which are the small membranous sacs found in the vestibule of the inner ear – involved in balance. Studies have demonstrated that balance is particularly affected by prenatal alcohol exposure (Riley and McGee 2005). A study by Kyllerman *et al.* (1985) demonstrated that children exposed to alcohol performed badly in balance tests with tremor and low general muscle tone. This included subjects that were not diagnosed with full-blown FAS. However, the results were more profound in children with full-blown FAS. Additionally, a study by Roebuck *et al.* (1998) published results that suggested that children exposed prenatally to alcohol relied more on somatosensory input, and when exposed to atypical input, these subjects displayed significant anterior-posterior body sway, and were unable to compensate using the visual or vestibular information that was available to them. It was suggested that these deficits were related to cerebellar abnormalities often found in children with prenatal alcohol exposure. It would be interesting to, in the future, correlate the volumetric results of the affected lobules of these particular subjects with vestibular-motor tests similar to those conducted by the above-mentioned studies to determine whether similar outcomes are achieved. In addition to its well-documented involvement in gait and balance, lobule X has also been associated with limbic functions (Balaban and Romero, 1998). The flocculonodular lobe, together with the vermis, has also been reported to be involved in emotion, with the proposal of a "limbic cerebellum" (Mills *et al.,* 2005).

Due to the fact lobule X has been associated with limbic functions, its involvement in functions of behaviour should be noted as well. Though the cerebellum has been reported to play a role in behavioural disorders (Riley and McGee, 2005; Guerri *et*

al., 2009; Kodituwakku, 2007), these findings have almost always involved the vermis and "paravermal" regions. Data suggests that even though many prenatally exposed children may be spared the physical consequences of prenatal alcohol exposure, they are still at risk for cognitive and behavioural problems. Furthermore, data also suggests that long-term deficits in adaptive and other behaviours may be present in individuals with prenatal alcohol exposure, even in the absence of cognitive deficits (Roebuck *et al.*, 1998). Cerebellar abnormalities have been reported in various behavioural disorders, and it could be possible that certain behavioural problems observed in specific disorders, like attention deficit and hyperactivity disorder (ADHD), for example, are caused by specific abnormalities in the cerebellum. ADHD has also been associated with problems of emotion (Barkley, 1997), and children with FASD have been noted to display behaviour associated with ADHD (Jacobson and Jacobson, 2002), in fact, a significant number of children diagnosed with FAS would qualify for a diagnosis of ADHD (Riley and McGee, 2005). Berquin *et al.* (1998) reported that male children with ADHD were found to have posterior inferior cerebellar lobe measures that were smaller than in control groups. These included measurements of lobules VIII, IX and X. Considering that this study found significant volume reductions for lobules IX and X, it may be fair to suggest a possible association between the behavioural problems that are often seen in both ADHD and FASD and these specific volume reductions.

The idea mentioned above does not only apply to ADHD-type behaviour. Carper and Courchesne (2000) have reported that patients with autism show an almost complete absence of a frontal lobe neurophysiological response that is related to attention. However, this same effect is seen in non-autistic adults that do not have frontal lobe damage, but have acquired cerebellar lesions from strokes or tumours. This is interesting to note, as children with FASDs have been reported to display problems with attention (Jacobson and Jacobson, 1999; 2002; Riley and McGee, 2005). This could, perhaps, suggest that some neurobehavioral problems, whether they be FASD-, ADHD- or autism-related, that involve attention deficiencies, may be caused by lesions or mal-development of the same area/s in the cerebellum that are interconnected to the area of the frontal lobe that is responsible for the above-mentioned neurophysiological attention-related response.

Volume differences involving Left Lobules I-V

Normalized volumes of left lobules I-V had a positive correlation with Proportion of drinking days per week around the time of conception (r = 0.23). This was the only region that demonstrated significant positive correlations with alcohol exposure. It was anticipated that, if some lobules showed significant decreases in volume, other lobules may appear to be significantly larger. The larger normalized volume observed for this region suggests that lobules I-V are not particularly sensitive to prenatal alcohol exposure, causing the normalized volume to appear larger. It is evident in Table 3.2 that there are no significant volume differences between diagnostic groups in this area, despite significant group differences in overall cerebellar cortex.

These results do not support the majority of publications that report decreases in lobule volumes of lobules I-V as a result of alcohol exposure. Animal studies have demonstrated that prenatal alcohol exposure produces Purkinje cell loss in lobules I-V (Goodlett *et al.,* 1990). This finding was further supported by an MRI study that looked at 9 children and young adults, reporting significantly smaller anterior lobe regions in the exposed subjects. They suggested that regionally specific Purkinje cell death also occurs in humans prenatally exposed to alcohol (Sowell *et al.,* 1996). Results of this study suggest that lobules I-V may be relatively spared.

Volume differences in right Vermis

Right Vermal volumes were significantly reduced (10^{th} percentile) when analysing lobule volume as a function of alcohol exposure, but not as a function of diagnosis. More specifically, normalized volume of the right vermis decreased with more drinking days per week averaged across pregnancy (r = -0.2) and this trend persisted after winsorisation (r = -0.19). Differences in vermal volume were significant when analysing as a function of diagnosis before normalizing for total cerebellar cortical volume. These results, however, did not remain significant after normalizing. Though the correlations are not strong, these results are in agreement with results published by O'Hare *et al.* (2005), which demonstrated that children that had been

prenatally exposed to alcohol had statistically significant volume reductions of the anterior vermis, posterior-inferior vermis, as well as displacement of the anterior and posterior-inferior regions of the vermis.

The vermis has been reported to be involved in various functions. It is a major target of the vestibular nuclei, and is a primary region for multimodal sensory and motor integration (Schmahmann, 2000). Not only has the vermis been found to be involved in sensorimotor functions, it has been associated with functions of the cognitive, emotional and behavioural domains. Boddaert *et al.* (2003) demonstrated that normal cognitive development in children with Dandy Walker Syndrome (DWS) was related to normal lobulation of the vermis. In the mentioned study, 14 out 20 subjects with DWS presented with normal cognitive abilities, and all 14 of them displayed normal vermal lobulation, whereas the remaining 6 subjects presented with abnormal cognitive abilities as well as abnormal vermis anatomy or associated vermal anomalies. This information further supports the idea of the vermis being significantly involved in the various cognitive problems associated with FASDs.

Anterior dysmorphology of the vermis has been negatively correlated with verbal learning and memory performance within alcohol-exposed groups (O'Hare *et al.,* 2005). Furthermore, a study by Moretti *et al.* (2002) proposed that acquired lesions of the vermis are associated with marked increase in reading mistakes, which can be seen in subjects with dyslexia. It was suggested that dyslexia caused by cerebellar impairment may be due to oculomotor alterations or disruption of cerebellar-encephalic projections, which connect the cerebellum, the vermis in particular, to the language system. Considering that children with FASDs have been reported to also perform poorly in reading, learning and language tasks (Mattson and Riley, 1998), these results could help strengthen the argument that vermal structures may be the parts of the cerebellum that are responsible for these specific impairments in reading and language that are often seen in children with FASDs.

The results of this study also support the findings in published reports of children prenatally-exposed to alcohol with emotional dysfunctions. Vermal regions project

to various parts of the brain that are believed to regulate mood; and, as previously mentioned, the vermis together with the fastigial nucleus and flocculonodular lobe (lobule X) may be an extention of Papez's emotion circuit, which one could call the "limbic cerebellum". Reduced functional activity of the cerebellar vermis has also been associated with emotions caused by early childhood stressful experiences (Teicher *et al.*, 2003). Furthermore, lesion studies have demonstrated that there are relationships between the vermis and emotion – the cerebellar cognitive-affective syndrome (CCAS) involves both emotional and cognitive deficits (Schmahmann, 2001a). This can be explained by the afferent and efferent connections of the cerebellum to the brainstem and limbic system, which lay the foundation for the cerebellar involvement in emotion as well as emotional disorders. However, it is not certain whether the cerebellum is specialized in specific forms of positive or negative emotional processes (Schutter and van Honk, 2005).

It is not only the sensory-motor, cognitive, behavioural and emotional aspects of prenatal alcohol exposure that can be connected to other pathologies to help one determine cerebellar involvement. It may be possible that some of the physical attributes found in children with FAS could be associated with structural abnormalities of the cerebellum, specifically, the vermis. Interestingly, holoprosencephaly has often been associated with facial malformations, which include those that are typically seen in children with FAS (Roebuck *et al.*, 1998). This could suggest that the facial features associated with FAS may be linked to an underlying brain structure. DWS, a condition that has been reported to present with facial anomalies (Hirsch *et al.*, 1984), has also been associated with holoprosencephaly, as well as hydrocephalus (Brodal and Hauglie-Hanssen, 1959) which, in turn, has also been reported in children with FASD (Roebuck *et al.*, 1998; Johnson *et al.*, 1984). It is also interesting to note that DWS is associated with the defective development or lobulation of the cerebellar vermis (Brodal and Hauglie-Hanssen, 1959; Steinlin, 2007), and this includes partial or total absence of the vermis (Kontopoulos *et al.*, 2008).

Volume differences in Lobule IX

Reductions in volume of lobule IX remained significant even after normalizing for total cerebellar cortex. This was the case when analysing these volumes as a function of diagnosis for both hemispheres combined and for the right hemisphere as a function of alcohol exposure (Tables 3.7 and 3.8). As a function of diagnosis, lobule IX in the FAS group was significantly smaller than the control group (T = -3.4). Increased alcohol exposure was significantly associated with smaller volumes in right lobule IX for all alcohol measures.

Literature discussing the functions associated with lobule IX is limited and, therefore, these results could not be discussed in as much detail as one would hope. Published literature suggests that lobule IX appears to be involved in functions belonging to the sensory domain. The cerebellum, with particular activation of lobule IX, has been reported to be involved in autonomic responses. A positron emission tomography (PET) study by Parsons *et al.* (2000) examined whether the cerebellum played a role in basic vegetative functions and the primal emotions that are generated. In the mentioned PET study, they found that, after allowing subjects to meet the point of maximum thirst, then, after drinking to satiation, lobule IX (amongst other lobules) became activated. They suggested that the activation seen in the PET scans of the relative lobules of the cerebellum were not due to the computation of thirst per se but, rather, it was activation seen during changes in thirst/satiation state when the relevant brain structures were "vigilant" and monitoring the sensory systems. Furthermore, Parsons *et al.* (2001), conducted a PET study to determine whether the cerebellum is involved in interoceptor-driven vegetative and autonomic functions involving air hunger. Lobule IX was reported to be activated during hypercapnia and air hunger. The activation of lobule IX in the study of air hunger may have been caused for the same reasons as mentioned in the study involving thirst. The activation seen may have been due to monitoring of the sensory systems involved in air hunger and re-exposure to oxygen, and not necessarily of the actual computation of needing air. This information suggests that lobule IX may be involved in functions that relate to 'primal' instincts of survival. Children with FASDs, specifically those with FAS, have been reported to need maternal or adult care and guidance, due to being vulnerable to maladaptive life

behaviours, even after ages of maturity have been reached (Streissguth *et al.*, 2004). It may be possible that part of the reason that children with FAS need additional parental guidance is not only related to their cognitive and emotional impairments but, if one considers the involvement of lobule IX in basic survival instincts, their instincts for survival and flourishing might be affected by autonomic sensory difficulties or the lack of monitoring autonomic sensory input.

4.2 OVERALL FINDINGS AND HOW THEY MAY RELATE TO DEVELOPMENT

The brain growth spurt is a transient period of rapid brain growth, which begins when adult neuronal number has been already largely achieved with the exception of certain cerebellar neurons, which have a peculiarly rapid growth rate over a shorter but coincident period of the brain growth spurt (Dobbing and Sands, 1973). The reasonably faster, yet slightly later, rate of growth of the cerebellum allows for the prediction that it may be particularly vulnerable during this fast rate of development. The above-mentioned study suggested that the structure within the brain that is growing the fastest would most probably show the greatest effects of growth restriction. The same study also stated that 5/6 of the human brain growth spurt is, in fact, postnatal. Considering that the cerebellum takes up a shorter time of the growth spurt than the rest of the brain, this means that the period of time that the cerebellum uses for rapid development *in-utero* must be rather late during gestation. This may explain why, in this particular study, the highest negative correlation found for volume of lobule IX and alcohol exposure was for the "across pregnancy" measure and not at "time of conception". Similarly, the only other correlation was found in the vermis with proportion of drinking days per week "across pregnancy" and not at "time of conception". The only correlation that was found "at time of conception" was the positive correlation with normalized volume of left lobules I-V. A possible reason for certain regions of the cerebellum being more vulnerable to the effects of prenatal alcohol exposure than others could be related to the timing of exposure as well as the rate and time at which different parts of the cerebellum develop. This may cause certain regions to be particularly vulnerable at particular stages.

An animal study by Thomas *et al*. (1998) presented data confirming that there are window periods of vulnerability of Purkinje cells to the effects of binge alcohol treatment. They also demonstrated that behavioural and neuroanatomical consequences of binge exposure are dependent on the developmental timing of the exposure. Another study by Goodlett *et al.* (1990) demonstrated that a single binge exposure during the growth spurt period was sufficient to produce neuron depletion, with the cerebellum being most severely reduced. Though these were animal studies, the identification of window periods is significant. Furthermore, it would be beneficial to conduct studies in the future that aim at identifying when during gestation these window periods would occur in humans. This would provide another possible way of predicting outcomes of prenatal alcohol exposure. Although this would be dependent on accurate maternal drinking histories, it would still be an additional method in aiding intervention strategies.

Considering the findings of this study along with the various possible explanations, it is hard to ignore the question of whether the structural abnormalities of the cerebellum in FASDs are primary problems or whether they are secondary atrophies that have been caused by problems of the cerebrum. Steinlin (2007) raised the point that the wide varieties of speech and neuropsychological problems that have been related to the cerebellum have not been unique to cerebellar functions – the cerebrum appears to be involved in these problems as well. They suggested that the primary function of the cerebellum may be in the area of learning, either during development or during rehabilitation processes.

4.3 LIMITATIONS

There are a few limitations of this study that need to be mentioned. Firstly, though using proportionate volume of lobule to total cerebellum is probably the best current method to address variation, caution was necessary since it was possible that there were dynamic developmental changes in the examined age range. This could possibly have resulted in the older participants in the sample not providing similar information to the younger participants in the sample – effects of age may require further investigation. Though the age range was not wide, it was necessary to

suggest this point as a possible limitation. Secondly, detailed statistical analyses were only preformed on normalised lobule volumes, and not on volumes in native space. Subgrouping resulted in a relatively small sample size for the FAS group. Reliability data was not available at time of dissertation submission and this, therefore, requires validation.

4.4 FUTURE DIRECTIONS

Though it makes sense to study cerebellar function by comparing individuals with certain disorders with those of control subjects, the disorders often lead to generalized developmental problems (Steinlin, 2007). It has been suggested that better models for analyses of cerebellar function would be those with focal cerebellar lesions.

Overall, the findings of this study have led to the following suggestions for future research:

- These results could be merged with other information that has been obtained for these subjects. This would include IQ test scores, behavioural test scores, eye-blink conditioning results, etc. In doing this, one could study correlations to examine structure-function relationships involving the cerebellum.
- The same MRI data can be used to look at hypoplasia of the cerebellar cortex. This could provide meaningful information because damage to the cerebellar cortex has been associated with lower verbal IQ and significant problems in judgement of duration (timing) (Mostofsky *et al.,* 2000).
- Results of this study could be compared in more detail to results of similar studies of the cerebellum and alcohol exposure. This could be useful for gaining a greater understanding of the 'normal' functioning cerebellum because "Although extensive research has substantially broadened the insights in the cognitive and affective role of the cerebellum, the precise nature of the cerebellar contribution to cognitive and affective regulation is not yet clear" (Baillieux *et al.,* 2008).

- Future work should investigate the normative proportion of lobule to total cerebellar volume across a large sample of healthy children ranging in the developmental age.
- Studies of this nature could assist with development of functionally defined cerebellar regions.

This volumetric study could be repeated using a larger sample size. A larger sample size may yield a larger range of significant results.

CHAPTER 5

CONCLUSION

Overall findings of this study suggest that prenatal exposure to alcohol disproportionately affects only specific lobules of the cerebellum of children. While various lobules demonstrated vulnerability to exposure to prenatal alcohol and smaller volumes, most of these effects no longer persisted after controlling for total cerebellar cortical volume. Normalized lobule volumes were analysed as functions of diagnosis as well as alcohol exposure. Lobules IX and X were affected when analysing normalized volumes as a function of diagnosis, with only the fetal alcohol syndrome diagnostic group being affected. Increased alcohol consumption, both at conception and throughout pregnancy, was associated with smaller volumes in right lobule IX. The strongest negative correlations were found with absolute alcohol per day across pregnancy rather than at time of conception. More drinking days per week across pregnancy was weakly associated with smaller right vermal volume.

This study was able to give a few details of what influences alcohol may have on the development of the cerebellum, which led to suggestions of which specific functions particular parts of the cerebellum may be responsible for. Furthermore, these results support findings of previous studies that have emphasized the significant involvement of prenatal alcohol exposure on the development of the vermis; and, considering data from various studies point to the great importance of the cerebellum, particularly the vermis, for the early development of cognitive functions, these results were considered meaningful. It may be that prenatal exposure to alcohol does not cause a fixed set of neurobehavioral outcomes but, instead, certain amounts of alcohol exposure at certain periods of development may cause particular cerebellar developmental problems; and, depending on what these anomalies in cerebellar development are, neurobehavioral outcomes will differ.

This study needed to also bear in mind that the division of the cerebellum into its subsections is a controversial topic on its own. Defining the lateral tips/edges of the

lobules was not always a straight forward task. At times, the lateral edges (especially at crus 1) became very blurred and were not easy to identify.

It is important to acknowledge that, in order to facilitate live morphometric analysis of the human cerebellum, specifically topography and volumetry, intensive studies are needed to better understand the human cerebellum and define its subsections. This needs to be accomplished in a uniform manner to provide a guide for the anatomic localization of cerebellar activation in fMRI studies. The ability to successfully define the specific subsections of the cerebellum and define the variations that occur amongst individuals may make it possible to specifically identify distinct functional subsystems that are found within the cerebellum. By achieving this, it could be possible to characterize acquired and developmental cerebellar abnormalities in terms of their effects on cerebellar volume and function (Makris *et al.,* 2003). However, the difficulty of this task is reflected by the great number of attempts that have been made in order to try to understand the morphological organization of the human cerebellum. Basic research in the field of FASDs might further the understanding of the relationships between cerebellar structure and function, given that so much can be learned about normal cerebellar functioning by studying abnormal cerebellar functioning.

The odds of escaping the adverse life outcomes of living with FASDs are increased 2- to 4-fold by receiving the diagnosis of FAS or FAE at an earlier age (Streissguth *et al.,* 2004) in order to provide stable living environments that are appropriate. Further studies of this nature would assist in eventually developing efficient methods for management of this preventable cause of CNS damage.

BIBLIOGRAPHY

Abel E, Sokol R (1987) Incidence of fetal alcohol syndrome and economic impact of FAS-related anomalies. Drug and alcohol dependence 19(1): 51-70.

Anand BK, Malhotra C L, Singh B, Dua S (1959) Cerebellar projections to limbic system. Journal of neurophysiology 22(4): 451-7.

Archibald S L, Fennema-Notestine C, Gamst A, Riley EP, Mattson SN, Jernigan TL (2001) Brain dysmorphology in individuals with severe prenatal alcohol exposure. Developmental medicine and child neurology 43(3): 148-54.

Autti-Rämö I, Autti T, Korkman M, Kettunen S, Salonen O, Valanne L (2002) MRI findings in children with school problems who had been exposed prenatally to alcohol. Developmental medicine and child neurology 44(2): 98-106.

Baillieux H, De Smet HJ, Paquier PF, De Deyn PP, Mariën P (2008) Cerebellar neurocognition: insights into the bottom of the brain. Clinical neurology and neurosurgery 110(8): 763-73.

Balaban CD, Romero GG (1998) A role of climbing fibers in regulation of flocculonodular lobe protein kinase C expression during vestibular compensation. Brain research 804(2): 253-65.

Barkley RA (1997) Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychological bulletin 121(1): 65-94.

Berquin PC, Giedd JN, Jacobsen LK, Hamburger SD, Krain AL, Rapoport JL, Castellanos FX (1998) Cerebellum in attention-deficit hyperactivity disorder: a morphometric MRI study. Neurology 50(4): 1087-93.

Boddaert N, Klein O, Ferguson N, Sonigo P, Parisot D, Hertz-Pannier L, Baraton J, Emond S, Simon I, Chigot V, Schmit P, Pierre-Kahn A, Brunelle F (2003) Intellectual prognosis of the Dandy-Walker malformation in children: the importance of vermian lobulation. Neuroradiology 45(5): 320-4.

Bowman RS, Stein LI, Newton JR (1975) Measurement and interpretation of drinking behavior. I. On measuring patterns of alcohol consumption. II. Relationships between drinking behavior and social adjustment in a sample of problem drinkers. Journal of studies on alcohol 36(9): 1154-72.

Brodal A, Hauglie-Hanssen E (1959) Congenital hydrocephalus with defective development of the cerebellar vermis (Dandy-Walker syndrome) clinical and anatomical findings in two cases with particular reference to the so-called atresia of the foramina of Magendie and Luschka. Journal of neurology, neurosurgery, and psychiatry 22(2): 99-108.

Brown RT, Coles CD, Smith IE, Platzman KA, Silverstein J, Erickson S, Falek A (1991) Effects of prenatal alcohol exposure at school age. II. Attention and behavior. Neurotoxicology and teratology 13(4): 369-76.

Campitelli G, Gobet F, Parker A (2005) Structure and stimulus familiarity: a study of memory in chess-players with functional magnetic resonance imaging. The Spanish journal of psychology 8(2): 238-45.

Carper RA, Courchesne E (2000) Inverse correlation between frontal lobe and cerebellum sizes in children with autism. Brain 123: 836-44.

Chernoff GF (1978) The fetal alcohol syndrome in mice: an animal model. Teratology 15(3): 223-9.

Clark CM, Li D, Conry J, Conry R, Loock C (2000) Structural and functional brain integrity of fetal alcohol syndrome in nonretarded cases. Pediatrics 105(5): 1096-9.

Cohen SB (1997) An evaluation of alternative PC-based software packages developed for the analysis of complex survey data. The American Statistician 51(3): 285-92.

Courchesne E, Press GA, Murakami J, Berthoty D, Grafe M, Wiley CA, Hesselink JR (1989) The cerebellum in sagittal plane--anatomic-MR correlation: 1. The vermis. American journal of roentgenology 153(4): 829-35.

Croxford J, Viljoen D (1999) Alcohol consumption by pregnant women in the Western Cape. South African medical journal 89(9): 962-5.

Dobbing J, Sands J (1973) Quantitative growth and development of human brain. Archives of disease in childhood 48(10): 757-67.

Ernhart CB, Morrow-Tlucak M, Sokol RJ, Martier S (1988) Underreporting of alcohol use in pregnancy. Alcoholism, clinical and experimental research 12(4): 506-11.

Fryer SL, McGee CL, Matt GE, Riley EP, Mattson SN (2007) Evaluation of psychopathological conditions in children with heavy prenatal alcohol exposure. Pediatrics 119(3): 733-41.

Goodlett CR, Marcussen BL, West JR (1990) A single day of alcohol exposure during the brain growth spurt induces brain weight restriction and cerebellar Purkinje cell loss. Alcohol 7(2): 107-14.

Goodlett CR, Horn KH, Zhou FC (2005) Alcohol teratogenesis: mechanisms of damage and strategies for intervention. Experimental biology and medicine 230(6): 394-406.

Guerri C, Bazinet A, Riley EP (2009) Foetal Alcohol Spectrum Disorders and alterations in brain and behaviour. Alcohol and alcoholism 44(2): 108-14.

Hastings CJ, Mosteller F, Tukey JW, Winsor CP (1947) Low Moments for Small Samples: A Comparative Study of Order Statistics. The Annals of Mathematical Statistics 18(3): 413-26.

Hirsch J, Pierre-kahn A, Renier D, Sainte-rose C, Hoppe-hirsch E (1984) The Dandy-Walker malformation. Journal of Neurosurgery 61(3): 515-522.

Hoyme HE, May PE, Kalberg WO, Kodituwakku P, Gossage JP, Trujillo PM, Buckley DG, Miller JH, Aragon AS, Khaole N, Viljoen DL, Jones KL, Robinson LK (2005). A practical clinical approach to diagnosis of fetal alcohol spectrum disorders: clarification of the 1996 institute of medicine criteria. Pediatrics 115(1): 39-47.

Jacobson JL, Jacobson SW (1999) Drinking moderately and pregnancy. Effects on child development. Alcohol research & health 23(1): 25-30.

Jacobson JL, Jacobson SW (2002) Effects of prenatal alcohol exposure on child development. Alcohol research & health 26 (4): 282-6.

Jacobson SW, Chiodo LM, Sokol RJ, Jacobson JL (2002) Validity of maternal report of prenatal alcohol, cocaine, and smoking in relation to neurobehavioral outcome. Pediatrics 109(5): 815-25.

Jacobson SW, Stanton ME, Dodge NC, Pienaar M, Fuller DS, Molteno CD, Meintjes EM, Hoyme HE, Robinson LK, Khaole N, Jacobson JL (2011) Impaired delay and trace eyeblink conditioning in school-age children with fetal alcohol syndrome. Alcoholism, clinical and experimental research 35(2): 250-64.

Jacobson SW, Stanton ME, Molteno CD, Burden MJ, Fuller DS, Hoyme HE, Robinson LK, Khaole N, Jacobson JL (2008) Impaired eyeblink conditioning in children with fetal alcohol syndrome. Alcoholism, clinical and experimental research 32(2): 365-72.

Johnson CA, Hill ID, Bowie MD (1984) Fetal alcohol syndrome with hydrocephalus. A case report. South African medical journal 65(18): 738-9.

Jones KL, Smith DW (1973) Recognition of the fetal alcohol syndrome in early infancy. Lancet 302: 999-1001.

Kelly SJ, Day N, Streissguth AP (2000) Effects of prenatal alcohol exposure on social behavior in humans and other species. Neurotoxicology and teratology 22(2): 143-9.

Kieran J A (2005) Barr's The Human Nervous System: an Anatomical Viewpoint. 8th ed. Lippincott Williams & Wilkins, Philadelphia.

Kodituwakku PW (2007) Defining the behavioral phenotype in children with fetal alcohol spectrum disorders: a review. Neuroscience and biobehavioral reviews 31(2): 192-201.

Kontopoulos EV, Quintero RA, Salihu HM, Bornick PW, Allen MH (2008) Dandy-Walker syndrome and monochorionic twins: insight into a possible etiological mechanism. The journal of maternal-fetal & neonatal medicine 21(11): 839-42.

van der Kouwe AJW, Benner T, Salat DH, Fischl B (2008) Brain morphometry with multiecho MPRAGE. NeuroImage 40(2): 559-69.

Kyllerman M, Aronson M, Sabel KG, Karlberg E, Sandin B, Olegård R (1985) Children of alcoholic mothers. Growth and motor performance compared to matched controls. Acta paediatrica Scandinavica 74(1): 20-6.

Larsell O, Jansen J (1972) The Comparative Anatomy and Histology of the Cerebellum. The Human Cerebellum, Cerebellar Connections, and Cerebellar Cortex. University of Minnesota Press, Minnesota.

Lupton C, Burd L, Harwood R (2004) Cost of fetal alcohol spectrum disorders. American journal of medical genetics 127C(1): 42-50.

Makris N, Hodge SM, Haselgrove C, Kennedy DN, Dale A, Fischl B, Rosen BR, Harris G, Caviness VS, Schmahmann JD (2003) Human cerebellum: surface-assisted cortical parcellation and volumetry with magnetic resonance imaging. Journal of cognitive neuroscience 15(4): 584-99.

Manning MA, Hoyme HE (2007) Fetal alcohol spectrum disorders: a practical clinical approach to diagnosis. Neuroscience and biobehavioral reviews 31(2): 230-8.

Mattson SN, Riley EP (1998) A review of the neurobehavioral deficits in children with fetal alcohol syndrome or prenatal exposure to alcohol. Alcoholism, clinical and experimental research 22(2): 279-94.

Mattson SN, Riley EP, Jernigan TL, Ehlers CL, Delis DC, Jones KL, Stern C, Johnson KA, Hesselink JR, Bellugi U (1992) Fetal alcohol syndrome: a case report of neuropsychological, MRI and EEG assessment of two children. Alcoholism, clinical and experimental research 16(5): 1001-3.

Mattson SN, Riley EP, Sowell ER, Jernigan TL, Sobel DF, Jones KL (1996) A decrease in the size of the basal ganglia in children with fetal alcohol syndrome. Alcoholism, clinical and experimental research 20(6): 1088-93.

Mills NP, Delbello MP, Adler CM, Strakowski SM (2005) MRI analysis of cerebellar vermal abnormalities in bipolar disorder. The American journal of psychiatry 162(8): 1530-2.

Moretti R, Bava A, Torre P, Antonello RM, Cazzato G (2002) Reading errors in patients with cerebellar vermis lesions. Journal of neurology 249(4): 461-8.

Mostofsky SH, Kunze JC, Cutting LE, Lederman HM, Denckla MB (2000) Judgment of duration in individuals with ataxia-telangiectasia. Developmental neuropsychology 17(1): 63-74.

Naidich TP, Duvernoy HM, Delman BN, Sorensen GA, Kollias SS, Haacke ME (2009) Duvernoy's Atlas of the Human Brain Stem and Cerebellum. 1st ed. SpringerWienNewYork, New York.

Norman AL, Crocker N, Mattson SN, Riley EP (2009) Neuroimaging and fetal alcohol spectrum disorders. Developmental disabilities research reviews 15(3): 209-17.

O'Hare ED, Kan E, Yoshii J, Mattson SN, Riley EP, Thompson PM, Toga AT, Sowell ER (2005) Mapping cerebellar vermal morphology and cognitive correlates in prenatal alcohol exposure. Neuroreport 16(12): 1285-90.

O'Reilly JX, Beckmann CF, Tomassini V, Ramnani N, Johansenberg H (2010) Distinct and Overlapping Functional Zones in the Cerebellum Defined by Resting State Functional Connectivity. Cerebral Cortex 20: 953-65.

Parsons LM, Denton D, Egan G, McKinley M, Shade R, Lancaster J, Fox PT (2000) Neuroimaging evidence implicating cerebellum in support of sensory/cognitive processes associated with thirst. Proceedings of the National Academy of Sciences of the United States of America 97(5): 2332-6.

Parsons LM, Egan G, Liotti M, Brannan S, Denton D, Shade R, Robillard R, Madden L, Abplanalp B, Fox PT (2001) Neuroimaging evidence implicating cerebellum in the experience of hypercapnia and hunger for air. Proceedings of the National Academy of Sciences of the United States of America 98(4): 2041-6.

Press GA, Murakami J, Courchesne E, Berthoty DP, Grafe M, Wiley CA, Hesselink JR (1989) The cerebellum in sagittal plane--anatomic-MR correlation: 2. The cerebellar hemispheres. American journal of roentgenology 153(4): 837-46.

Riley EP, McGee CL (2005) Fetal alcohol spectrum disorders: an overview with emphasis on changes in brain and behavior. Experimental biology and medicine 230(6): 357-65.

Roebuck TM, Mattson SN, Riley EP (1998) A review of the neuroanatomical findings in children with fetal alcohol syndrome or prenatal exposure to alcohol. Alcoholism, clinical and experimental research 22(2): 339-44.

Roebuck TM, Simmons RW, Mattson SN, Riley EP (1998) Prenatal exposure to alcohol affects the ability to maintain postural balance. Alcoholism, clinical and experimental research 22(1): 252-8.

Roebuck-Spencer TM, Mattson SN (2004) Implicit Strategy Affects Learning in Children With Heavy Prenatal Alcohol Exposure. Alcoholism: Clinical & Experimental Research 28 (9): 1424-31.

Schmahmann JD, Doyon J, McDonald D, Holmes C, Lavoie K, Hurwitz AS, Kabani N, Toga A, Evans A, Petrides M (1999) Three-dimensional MRI atlas of the human cerebellum in proportional stereotaxic space. NeuroImage 10: 233-60.

Schmahmann, Jeremy D. 2000. The role of the cerebellum in affect and psychosis. Journal of Neurolinguistics 13: 189-214.

Schmahmann JD (2001a) The cerebrocerebellar system: anatomic substrates of the cerebellar contribution to cognition and emotion. International Review of Psychiatry 13(4): 247-260.

Schmahmann JD (2001b) The cerebellar cognitive affective syndrome: clinical correlations of the dysmetria of thought hypothesis. International Review of Psychiatry 13(4): 313-322.

Schutter DJLG, van Honk J (2005) The cerebellum on the rise in human emotion. Cerebellum 4(4): 290-4.

Sowell ER (2002) Mapping Cortical Gray Matter Asymmetry Patterns in Adolescents with Heavy Prenatal Alcohol Exposure. NeuroImage 17(4): 1807-19.

Sowell ER, Jernigan TL, Mattson SN, Riley EP, Sobel DF, Jones KL (1996) Abnormal development of the cerebellar vermis in children prenatally exposed to alcohol: size reduction in lobules I-V. Alcoholism, clinical and experimental research 20(1): 31-4.

Sowell ER, Thompson PM, Mattson SN, Tessner KD, Jernigan TL, Riley EP, Toga W (2001) Voxel-based morphometric analyses of the brain in children and adolescents prenatally exposed to alcohol. Neuroreport 12(3): 515-23.

Sowell ER, Thompson PM, Mattson SN, Tessner KD, Jernigan TL, Riley EP, Toga AW (2002) Regional brain shape abnormalities persist into adolescence after heavy prenatal alcohol exposure. Cerebral cortex 12(8): 856-65.

Spadoni AD, McGee CL, Fryer SL, Riley EP (2007) Neuroimaging and fetal alcohol spectrum disorders. Neuroscience and biobehavioral reviews 31(2): 239-45.

Spottiswoode BS, Meintjes EM, Anderson AW, Molteno CD, Stanton ME, Dodge NC, Gore JC, Peterson BS, Jacobson JL, Jacobson SW (2011) Diffusion tensor imaging of the cerebellum and eyeblink conditioning in fetal alcohol spectrum disorder. Alcoholism, clinical and experimental research 35(12): 2174-83.

Steinlin M (2007) The cerebellum in cognitive processes: supporting studies in children. Cerebellum 6(3): 237-41.

Streissguth AP, Aase JM, Clarren SK, Randels SP, LaDue RA, Smith DF (1991) Fetal alcohol syndrome in adolescents and adults. JAMA : the journal of the American Medical Association 265(15): 1961-7.

Streissguth AP, Bookstein FL, Barr HM, Sampson PD, O'Malley K, and Young JK (2004) Risk factors for adverse life outcomes in fetal alcohol syndrome and fetal alcohol effects. Journal of developmental and behavioral paediatrics 25(4): 228-38.

Swayze VW, Johnson VP, Hanson JW, Piven J, Sato Y, Giedd JN, Mosnik D, Andreasen NC (1997) Magnetic Resonance Imaging of Brain Anomalies in Fetal Alcohol Syndrome. Pediatrics 99(2): 232-240.

Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP, Kim DM (2003) The neurobiological consequences of early stress and childhood maltreatment. Neuroscience and biobehavioral reviews 27(1-2): 33-44.

Thomas JD, Goodlett CR, West JR (1998) Alcohol-induced Purkinje cell loss depends on developmental timing of alcohol exposure and correlates with motor performance. Brain research 105(2): 159-66.

Thomas JD, Warren KR, Hewitt BG (2010) Fetal alcohol Spectrum Disorders. Alcohol Research & Health 33(1-2): 118-26.

Tisdall MD, Hess AT, Reuter M, Meintjes EM, Fischl B, van der Kouwe AJ (2012) Volumetric navigators for prospective motion correction and selective reacquisition in neuroanatomical MRI. Magnetic resonance in medicine 68(2): 389-99.

Voogd J, Glickstein M (1998) The anatomy of the cerebellum. Trends in neurosciences 21(9): 370-5.

Woodruff-Pak DS,Goldenberg G, Downey-Lamb MM, Boyko OB, Lemieux SK (2000) Cerebellar volume in humans related to magnitude of classical conditioning. Neuroreport 11(3): 609-15.

Woods RP (2003) Multitracer: a Java-based tool for anatomic delineation of grayscale volumetric images. NeuroImage 19(4): 1829-34.

Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, Gerig G. (2006) User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. NeuroImage 31(3): 1116-28.