Social cognition in South African children with Fetal Alcohol Spectrum Disorders

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Abstract

Research on the social-cognitive profile of individuals with prenatal alcohol exposure (PAE) has confirmed poorer social skills in these children compared to healthy controls, independent of overall cognitive functioning. However, although children with fetal alcohol spectrum disorders (FASD) are known to have deficits in social-cognitive function, very little is known about the mechanisms underlying these impairments. I investigated social cognition in children with FASD by assessing Theory of Mind and emotion recognition ability as potential determinants of impaired social cognition, behaviourally and using neuroimaging. Study I showed that children aged 9-11 years (*N*=63) with fetal alcohol syndrome (FAS) and partial FAS performed more poorly on the Reading the Mind in the Eyes test, after controlling for IQ and executive function, suggesting difficulty in inferring people's mental states. Study II investigated the ability of 9-12 year old children (*N*=88) to read people's facial emotions because this more basic level of social cue processing was considered a possible precursor to the impairments seen in Study I. An affective appraisal and working memory (WM) task (1-back and 2-back) was administered. Groups performed well on the 1-back, indicating ability to meet WM demands of the affective appraisal task. No behavioural group differences were shown on the affective appraisal task, which confirmed the suitability of this task to identify possible differences in neuronal activation, which I investigated in Study III. Analyses of these fMRI data on 64 children aged 9-14 years showed that participants performed well on the relatively simple affective appraisal task. However, greater cortical activation was shown in exposed children when processing positive but less when processing negative facial expressions. These data demonstrate that heavy PAE alters activation within a cortical affective processing network. Because we know that children with FASD have alcohol-related social-cognitive impairments (Study I), differences in cortical activation may suggest that

when children with FASD need to appraise affect in more challenging contexts, as in dynamic social interactions, they are likely to have greater difficulties. These data are consistent with two ideas: a) that alcohol-exposed children have difficulty appraising negative emotions and b) that difficulty contributes to the clinically described trouble these children have in "reading" facial social cues. If this is true, then an intervention program that improves the ability of these children to appraise negative emotions will likely (a) improve their ability to correctly interpret the context of their social interactions; (b) contribute to developing mental representations of an appropriate reaction to a given situation and (c) positively affect the various evaluation processes during social information processing, which in turn are imperative to social-cognitive functioning.

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List of Abbreviations

Abbreviation	Description
AA	Absolute Alcohol
ADHD	Attention deficit hyperactivity disorder
ANCOVA	Analysis of Covariance
ANOVA	Analysis of variance
AR	NEPSY-II affect recognition task
ARBD	Alcohol-related birth defects
ARND	Alcohol-related neurodevelopmental disorder
AS	Asperger's syndrome
ASC	Affective Social Competence
ASD	Autism spectrum disorders
β	Beta coefficient
BOLD	Blood oxygenation level dependant
BPD	Borderline personality disorder
CDRL	Child Development Research Laboratory
CD	Conduct Disorder
CNS	Central nervous system
CUBIC	Cape Universities Brain Imaging Centre
DBD	Disruptive Behaviour Disorders Inventory
EF	Executive function
EHI	Edinburgh Handedness Test
η	Eta – effect size coefficient
FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorders
fMRI	Functional magnetic resonance imaging
HE	Heavily exposed
IOM	Institute of Medicine
IQ	Intelligence quotient
JSAIS	Junior South African Intelligence Scale

M	Mean
N	Total number of cases
n	Number of cases in a subsample (e.g., specific participant group)
K-SADS-PL	Schedule for Affective Disorders and Schizophrenia for School Aged Children (6-18 years) Lifetime Version
LSD	Least significant difference
MRI	Magnetic resonance imaging
mm^3	Millimetres cubed
ODD	Oppositional Defiant Disorder
oz.	Ounces
p	p-value
PAE	Prenatal alcohol exposure
PFAS	Partial fetal alcohol syndrome
RME	Reading the Mind in the Eyes
SD	Standard deviation
SES	Social economic status
SIP	Social Information Processing
SPM	Statistical Parametric Mapping
SPSS	IBM SPSS statistical analysis software
ToM	Theory of Mind
UCT	University of Cape Town
US	United States
WISC-IV	Wechsler Intelligence Scale for Children – 4^{th} edition
WM	Working memory

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INTRODUCTION

This dissertation is submitted for the degree of Doctor of Philosophy at the University of Cape Town. During the dissertation process, I identified gaps in the literature and formalised the testable theories and hypotheses and personally conducted all the data collection and data analyses, which culminated in my writing up of the results, findings and interpretations presented in this doctoral dissertation. The research described herein was conducted under supervision of Dr Susan Malcolm-Smith and co-supervision of Associate Professor Kevin Thomas in the Department of Psychology, University of Cape Town, and under mentorship and guidance of Professors Sandra Jacobson and Joseph Jacobson in the Department of Psychiatry and Behavioural Neuroscience, Wayne State University, Michigan.

The central theme of this doctoral dissertation is an exploration of social cognition in South African children with fetal alcohol spectrum disorders (FASD). Previous research suggests that children prenatally exposed to alcohol have impairments in social skills and peer relations, which affect their overall social-cognitive functioning. However, little is known about the mechanisms related to prenatal alcohol exposure that underlie these highly debilitating deficits in social competence. In the Western Cape province of South Africa, the prevalence of FASD is among the highest in the world; thus, investigating the underlying causes of social-cognitive impairments in this population is especially important. To unveil the potential contributing factors to the social-cognitive impairments seen in FASD, I employed a theoretical social information processing and affective social competence model.

In *Chapter One* I begin with a general review of the literature on FASD. Its purpose is to provide an overview of the effects of prenatal alcohol exposure on the developing fetus and the long-term and irreversible developmental consequences thereof. First, the various subtypes and the diagnostic criteria of FASD are discussed, along with a short history, to

provide a fundamental understanding of the disorder. Second, the behavioural and cognitive profile of individuals with FASD are examined based on prior research. Third, FASD research within the South African context is highlighted.

Chapter Two provides an overview of the theoretical frameworks upon which the research is built. This review examines social information processing and affective social competence in relation to affective processing and Theory of Mind and how impairments within these integrated systems may relate to known deficits in FASD. The various socioemotional constructs under investigation are outlined, and their potential relevance as they pertain to the FASD population is discussed.

I then move on to explain the context and general methods of the current research project in *Chapter Three*. An important note here, is that because participants for all studies were recruited from the same longitudinal cohort, there is some repetition across the three studies pertaining to recruitment, ethics and procedure. References to previous sections in the dissertation may be made in such instances.

In *Chapter Four*, I present the findings of Study I, which was conducted to examine potential difficulties in Theory of Mind ability in order to identify possible relations to social-cognitive impairments. The findings of Study I have been published and are in press at *Alcoholism: Clinical and Experimental Research*, and parts of the article have been included as Chapter Four of this dissertation. I designed this study, collected and analysed all the data, and then wrote up the findings from this study. This article was co-authored by Susan Malcolm-Smith, Kevin G. F. Thomas, Joseph L. Jacobson, Neil Dodge, Christopher D. Molteno, Ernesta M. Meintjes and Sandra W. Jacobson, given their consultation and contributions. Professor Visser, the Chair of the UCT Doctoral Degrees Board, has approved

my request to include the publication as part of this dissertation. For dissertation purposes, the manuscript has been modified to provide more detail.

Chapter Five summarises the findings of Study II, which assessed performance on tasks of affective appraisal and working memory. The behavioural output of each participant group is analysed and discussed and the rationale for Study III is presented in light of these findings.

In *Chapter Six*, I present the findings of Study III, the neuroimaging study. Here, children with FASD had to perform the same affective appraisal task as used in Study II, but did so during functional magnetic resonance imaging in a scanner. The purpose of this study was to investigate potential between group differences in neuronal activation in cortico-limbic structures during affective appraisal. The imaging methods employed in this study are discussed, and the behavioural data compared to those from Study II. Results of the preprocessed imaging data at the individual and group level were examined by diagnostic group, using a regions of interest analysis.

The final chapter, *Chapter Seven*, is an overall discussion of the findings of all three studies. In this chapter, I consider the key findings from each study and discuss them in relation to previous literature and the current theoretical framework, and in light of the effects of prenatal alcohol exposure. Limitations of the current research project and directions for future research are also presented before closing with final remarks and commentary.

Together, the chapters described above will demonstrate how the study of social cognition in FASD is pertinent, especially within the South African context. To my knowledge, this is the first research project to investigate the mechanisms of social cognition in FASD using the current approach and method. It is also the first study to examine social cognition in children with FASD in South Africa. Moreover, these children were recruited

prospectively, providing the opportunity to examine differences by FASD diagnosis as well as by continuous measures of alcohol exposure collected during pregnancy, independent of FASD diagnosis. Furthermore, this is the first neuroimaging study to investigate potential differences in neuronal activation during affective appraisal in the FASD population. The innovative methodological approaches adopted across the three studies, make the findings of this dissertation a novel contribution to the FASD literature. This is true, not only for the South African research context but also because of its contribution to the knowledge regarding FASD and developmental prenatal alcohol exposure in general.

CHAPTER ONE: Fetal Alcohol Spectrum Disorders

Introduction

The adverse and detrimental effects of prenatal alcohol exposure (PAE) on the developing brain and body have been recognised as a major global public health issue (Riley & McGee, 2005; Sokol, Delaney-Black, & Nordstrom, 2003). PAE is considered to be the leading cause of birth defects and of cognitive and developmental impairments, more so than any other biological (non-genetic) determinants (May & Gossage, 2011). In the United States (US), the estimated number of newborns with fetal alcohol spectrum disorders (FASD) is 40 000 per year (Lupton, Burd, & Harwood, 2004). In contrast, the Autism Society of America (2009) estimates the diagnosis of new autism cases in the US to be around 24 000 each year, which highlights the significance of understanding PAE. Although a vast range of associated neurobehavioural and cognitive deficits have been demonstrated and the damage has been recognised as irreversible, PAE is entirely preventable.

However, it was only in the late 1960s that the connection between maternal alcohol consumption during pregnancy and the problematic profiles of children born to these mothers was first identified (Lemoine, Harousseau, Borteyru, & Menuet, 1968). A few years later, when a definable syndrome was recognised, the term fetal alcohol syndrome (FAS) was coined (Jones & Smith, 1973; Jones, Smith, Ulleland, & Streissguth, 1973). At that time, the notion of a variable yet specific pattern of malformation and impairments related to PAE became evident and was first formalised.

Teratogenicity of Alcohol

Alcohol is a teratogen that interferes with the normal development of the fetus. Of all the substances that can be abused by a woman during pregnancy, alcohol is one of the most

serious, resulting in long-term negative effects and severe and irreversible damage. The teratogenic effects of alcohol vary depending on several factors including amount of alcohol consumed (e.g., Jacobson & Jacobson, 1999), the developmental processes occurring at the time of gestation (Medina, 2011; Sulik, Johnston, & Webb, 1981), and genetic differences (Dodge, Jacobson & Jacobson, 2014; Jacobson, Carr et al., 2006; McCarver, Thomasson, Martier, Sokol, & Li, 1997; Viljoen et al., 2001; Warren & Li, 2005). A dose-dependent relation of alcohol exposure and pattern of brain dysmorphogenesis has been shown in the first 3-6 weeks of prenatal development (Konovalov, Kovetsky, Bobryshev, & Ashwell, 1997), and effects of prenatal exposure can already be seen during the neonatal period (Taylor et al., 2015).

The effects of PAE on the developing fetus manifest themselves in the disruption of growth progression and neuronal functioning (Caley, Kramer, & Robinson, 2005; O'Leary, 2004). During the first trimester alcohol exposure can affect neural tube and crest formation resulting in brain malformation, such as microcephaly (Miller, 1996) and hydrocephaly (Webster, Walsh, Lipson, & McEwen, 1980). The characteristic facial morphology, most prominent in FAS, arises due to alcohol exposure in the first trimester (Sulik et al., 1981). PAE during the second and third trimester can result in the abnormal migration of cortical neurons and impairments in brain plasticity, respectively (Ikonomidou et al., 2000; Miller & Robertson, 1993). Although amount and frequency of alcohol exposure is related to the severity of the resulting syndrome (Jacobson & Jacobson, 1999), the exact quantity and timing of consumption necessary to produce a specific subtype of the syndrome has not yet been determined. However, functional impairment in individuals with a confirmed history of PAE is associated with binge drinking behaviour (Willford, Richardons, Leech, & Day, 2004) of at least once per week during pregnancy (Jacobson & Jacobson, 1999). Because there is no

known safe amount of prenatal exposure, recommendations regarding the safest behaviour for pregnant women or for women planning to fall pregnant is abstinence.

In addition to overall brain size and shape abnormalities, neuroimaging studies have noted structural and functional anomalies in children with FASD. Neuroanatomical differences have been seen specifically in the cerebellum, basal ganglia, frontal lobes, hippocampus and corpus callosum (for review, see Coles & Li, 2011; Lebel, Roussotte, & Sowell, 2011; Riley & McGee, 2005; Spadoni, McGee, Fryer, & Riley, 2007).

The term Fetal Alcohol Spectrum Disorders

The teratogenic insult of PAE results in a large number of physical, behavioural and cognitive deficits that can be collectively observed and are described under the umbrella term FASD. A woman must consume alcohol during her pregnancy for some form of FASD to emerge. A number of factors contribute to the severity of the disorder. These factors include the pattern of maternal drinking, such as timing of exposure (i.e., which gestational phase) and dosage of alcohol exposure (i.e., the frequency and amount) (Jacobson et al., 2008), as well as a variety of maternal risk factors, such as genetics, maternal age and nutrition, and post-natal infections (e.g., Chiodo et al., 2010; Jacobson, Jacobson, Sokol, & Ager, 1998; Jacobson, Carr et al., 2006; Jacobson, Jacobson, Sokol, Chiodo, & Corobana, 2004; May et al., 2008; May & Gossage, 2011).

The great variability in exposure results in an array of deficits ranging from mild to severe. Severity along the FASD spectrum is linked to greater alcohol consumption and is also related to age, maternal stature and socioeconomic status (SES). Older and smaller mothers of lower SES have been shown to have children who are more severely affected (Carter, Jacobson, Sokol, Avison, & Jacobson, 2013; May et al., 2008; Jacobson, Jacobson, Sokol, & Ager, 1998; Jacobson et al., 2004). The combination of effects of PAE occur along

the FASD continuum, and various maternal, physical (including anomalies of the central nervous system, facial and growth dysmorphology) and behavioural factors of the child are considered during the diagnostic process. Along the spectrum, the resulting phenotypes are grouped into four subtypes: FAS, partial FAS (PFAS) and alcohol-related effects, differentiated as either alcohol-related birth defects (ARBD) or alcohol-related neurodevelopmental disorder (ARND).

Diagnosis of Fetal Alcohol Spectrum Disorders

Diagnosis of FASD is complex and is governed by strict criteria outlined by the Institute of Medicine (IOM) of the National Academy of Sciences in 1996, and revised in 2005 (Hoyme et al., 2005). Detailed diagnostic schemes for FASD have also been proposed using the Canadian guidelines (Chudley et al., 2005) and in a 4-digit code developed by Astley and Clarren (2000). The IOM-Revised criteria were used in the present studies and will be described here. According to these guidelines, the diagnostic process requires evidence pertaining to four categories: (a) maternal alcohol consumption during pregnancy; (b) presence of a specific pattern of facial anomalies; (c) prenatal or postnatal growth retardation; and (d) abnormal brain growth or morphogenesis, i.e., anomalies within the central nervous system (CNS). For the diagnosis of a particular FASD subtype, a specific combination of these features needs to be identified, which allows for an individual to be categorized accordingly.

FAS is the most severe of the subtypes as it displays the entire phenotype, which is characterised by the distinctive craniofacial dysmorphology, including short palpebral fissures, a thin upper lip and a flat or smooth philtrum; small head circumference; and growth retardation. During diagnosis, great care needs to be taken to differentiate similar physical anomalies between FAS and other genetic and malformation syndromes (e.g., Williams

syndrome), which can occur with but are unrelated to PAE (Hoyme et al., 2005). If a child has all the characteristics of FAS and disorders that may mimic alcohol-related anomalies have been ruled out, maternal alcohol exposure is assumed and does not have to be confirmed for an FAS diagnosis.

PFAS is diagnosed when at least two of the facial anomalies are present together with small head circumference or growth retardation, or cognitive/behavioural developmental abnormalities that cannot be explained by other predispositional factors, such as genetics, family background or environment (Hoyme et al., 2005). Maternal alcohol use should be confirmed.

ARBD is diagnosed in individuals with confirmed maternal alcohol exposure and who have typical faces and show alcohol-related congenital major or minor structural anomalies (Hoyme et al., 2005). Diagnosis of ARND requires confirmation of maternal alcohol exposure and evidence of brain growth abnormality or morphology and alcohol-related behavioural and cognitive deficits that cannot be otherwise explained. Hence, even individuals that lack the distinctive dysmorphic features or have been prenatally exposed to low-to-moderate levels of alcohol may present with cognitive and behavioural deficits (Mattson, Riley, Gramling, Delis & Jones, 1998; Jacobson et al., 2004).

Behavioural Profile of Individuals with Fetal Alcohol Spectrum Disorders

Apart from the physical and dysmorphic features, the effects of PAE also manifest themselves in a wide-range of behavioural and cognitive deficits (Mattson, Crocker & Nguyen, 2011). These can vary considerably between individuals and occur in a continuum along the spectrum. A wide range of impaired cognitive and behavioural domains have been identified and well documented. These include overall deficits in intelligence (IQ), attention, learning, memory, executive function (EF) and social emotion processing (e.g., Burden,

Jacobson, Sokol & Jacobson, 2005; Carmichael Olson, Feldman, Streissguth, Sampson & Bookstein, 1998; Coles, Lynch, Kable, Johnson, & Goldstein, 2010; Day, Helsel, Sonon, & Goldschmidt, 2013; Jacobson et al., 2004, 2008; Kodituwakku, 2007; Kully-Martens, Denys, Treit, Tamana, & Rasmussen, 2012; Lewis et al., 2015; Mattson et al., 2011; Rasmussen, 2005; Streissguth, Barr, & Sampson, 1990; Streissguth et al., 1994).

IQ and FASD. Poorer general intellectual ability among children with FASD is one of the most common deficits associated with PAE. Although this impairment can be present in children without the cardinal facial features and in the absence of growth retardation, individuals with FAS generally appear to be more severely impaired than individuals with low levels of exposure (Dalen, Bruaroy, Wentzel-Larsen, & Laegreid, 2009; Mattson, Riley, Gramling, Delis, & Jones, 1997). In that regard, the estimated average IQ for North American individuals with FAS is 70 (Streisguth et al., 1991) whereas individuals who lack the dysmorphic features who have an average IQ of 80 (Mattson et al., 1997). In a study of South African children with FASD (Dodge et al., 2009), children with FAS had an average IQ of 55.9 (SD=9.5) and heavily exposed nonsyndromal children of 66.5 (SD=10.6) in comparison to a control group whose IQ was 77.3 (SD=10.2). A study on effects of moderate PAE (Jacobson et al., 2004) found no relation of PAE to IQ for the sample as a whole. However, when maternal age was considered as a moderator of the effect of alcohol, an alcohol effect on IQ emerged among children born to older mothers (≥ 30 years of age at time of delivery).

EF and FASD. A major cognitive domain often considered the primary cause of other secondary impairments in FASD is EF (e.g., Streissguth et al., 1990). Thus, a wide array of EF domains has been extensively investigated in individuals with FASD, and a broad range of EF deficits have been described in relation to PAE in children and adolescents. These include cognitive flexibility (response inhibition, fluency and set shifting), planning and working

memory. Importantly, in individuals with PAE, these impairments in EF are not merely associated with poorer IQ but may affect cognitive functioning independent of level of intelligence (Rasmussen, 2005). Poor cognitive flexibility in individuals with FASD has been demonstrated on several neuropsychological tests including tasks of verbal fluency (Jacobson, Jacobson, Sokil, Chiodo et al., 1998; Kodituwakku et al., 1995), design fluency (Schonfeld, Mattson, Lang, Delis, & Riley, 2001), visual-spatial verbal fluency (Mattson, Goodman, Caine, Delis & Riley, 1999) and set-shifting (Coles et al., 1997; Kodituwakku, Handmaker, Cutler, Weathersby, & Handmaker, 1995). Children with PAE also demonstrated poorer response inhibition and planning ability than healthy controls (Mattson et al., 1999; Kodituwakku, et al., 1995).

Attention and working memory in FASD. Some studies have reported that children with FASD have poor sustained attention (the ability to remain focused on a task over a certain time span; Nanson & Hiscock, 1990 but see Coles et al., 1997) and deficits in working memory performance (the ability to hold in mind and manipulate new and stored information; Diwadkar et al., 2013; Jacobson et al., 1998b; Rasmussen, Soleimani & Pei, 2011; Streissguth et al., 1990). Previous research suggests that the sustained attention deficit related to PAE is secondary to impairments in EF (Jacobson & Jacobson, 1999).

Working memory seems to be the attentional domain most affected by PAE and is affected independently of IQ (Burden et al, 2005; Connor, Sampson, Bookstein, Barr & Steissguth, 2000). A study by Diwadkar et al. (2013) demonstrated that although children with FASD perform as well as typically developing children on less complex working memory tasks, they recruit different brain regions to complete these tasks. These findings also showed that individuals with FAS/PFAS had different neural activation patterns compared to

nonsyndromal heavily exposed children, and that this may possibly be related to differences in dosage and timing of exposure.

Research has also shown a high co-morbidity between FASD and attention deficit hyperactivity disorder (ADHD). However, the etiology, mechanisms, and presentation of ADHD in children with FASD appear to be different from those seen in individuals with ADHD only. Inattention symptoms appear to emerge earlier in children with FASD, and hyperactivity is less frequent than inattention (Coles, 2011; O'Malley & Nanson, 2002).

Memory and FASD. A large body of research characterises specific aspects of memory and learning impairment in individuals with FASD. Within this domain, encoding of new verbal information appears to be the area most affected by PAE, while retrieval of learnt information is considered relatively spared (Kerns, Don, Mateer, & Streissguth, 1997; Kaemingk, Tanner Halverson, & Mulvaney, 2003; Mattson, Riley, Delis, Stern, & Jones, 1996; Mattson et al., 2011). However, a recent study showed that retrieval of learnt information is also impaired, but that these deficits in memory retrieval were no longer evident after adjusting for initial learning (Lewis et al., 2015). Impairment in other modalities of memory, such as visual-spatial learning and memory (Gray & Streissguth, 1990; Kaemingk & Tanner Halverson, 2000; Uecker & Nadel, 1996), spatial navigation (Hamilton, Kodituwakku, Sutherland, & Savage, 2003) and prospective memory (Lewis et al., under review) have also been documented in individuals with heavy PAE.

Numerosity and FASD. Arithmetic is among the domains most affected by PAE (e.g., Goldschmidt, Richardson, Stoffer, Geva, & Day, 1996; Jacobson et al., 2004; Howell, Lynch, Platzman, Smith, & Coles, 2006; Sampson, Streissguth, Barr, & Bookstein, 1989; Streissguth, Bookstein, Sampson, & Parr, 1995). Following the Dehaene (2003) number-processing model, alcohol-related arithmetic deficits have been examined in terms of their number

processing components, specifically mental representation and manipulation of numbers and quantities. Such alcohol-related deficits in numerosity can already be observed during infancy and have been shown to be independent of IQ (Jacobson et al., 2003). Neuroimaging studies have also demonstrated that in comparison to controls, children with FASD recruit a broader range of brain regions to complete simple tasks related to number processing (Meintjes et al., 2010; Woods, Meintjes, Molteno, Jacobson & Jacobson, 2015).

The above review outlines a range of major deficits across a number of cognitive domains, highlighting the debilitating developmental impairments and adverse consequences of PAE. However, children with FASD are also known to have deficits in social cognition, a cognitive domain that has received considerably less attention in the literature despite causing an array of problems in day-to-day interactions. Impairment in social cognition adversely impacts social skills and influences behaviour, which in turn also affects peer relations and interpretation of the social environment.

Social cognition in FASD. Children with FASD are often described as having trouble with social appropriateness or social judgment, or as overly friendly (Bishop, Gahagan, & Lord, 2007; Greenbaum, Stevens, Nash, Koren, & Rovet 2009; Mattson & Riley, 2000). Caregiver and teacher reports on individuals prenatally exposed to alcohol confirm that such individuals do indeed demonstrate poorer social competence (Brown et al., 1991; Greenbaum et al., 2009; Roebuck, Mattson, & Riley, 1999; Schonfeld, Paley, Frankel, & O'Connor, 2006). Longitudinal studies have demonstrated that social skills deficits become more pronounced with age and persist into adulthood (Carmichael Olsen et al., 1997; Streissguth et al., 1991). These life-long deficits in social competency may contribute to the development of secondary FASD-related disabilities, such as mental health problems and substance abuse (Streissguth, Barr, Kogan, & Bookstein, 1996).

Impairment in EF appears to mediate some of the FASD-related deficits in social behaviour and some research has suggested a causal relation between impairment in EF and the social behaviour problems typical of children with PAE (McGee, Fryer, Bjorkquist, Mattson, & Riley, 2008; Schonfeld et al., 2006). Within the social-cognitive domain, children with FASD have been shown to have deficits in tasks of emotion processing and aspects of Theory of Mind (ToM; Greenbaum et al., 2009; Rasmussen, Wyper, & Talwar, 2009). Whereas the profile of the social skills impairments in individuals with PAE is well documented, the mechanisms underlying these deficits are poorly understood. Thus, further investigation into social functioning-related domains, such as ToM including affective processing, may provide insight into the complex underlying processes that prohibit normal social-cognitive functioning following PAE. A more detailed overview of the social-cognitive impairments in children with FASD and the overall rationale and approach of the current research project is discussed in *Chapter Two.*

In addition to the neuropsychological consequences of PAE, marked behavioural impairments have been associated with the effects of alcohol exposure, including increased externalising and internalising behavioural problems (Mattson & Riley, 2000). It has been extensively demonstrated that PAE increases the risk for psychiatric disorders, such as mood and conduct disorders, impulsivity problems, impaired moral decision-making, higher incidence of delinquent behaviour and substance abuse and higher rates of suicide among individuals with FASD (Disney, Iacono, McGue, Tully, & Legrand, 2008; Fryer, McGee, Matt, Riley, & Mattson, 2007; O'Connor & Paley, 2006; Roebuck et al., 1999; Schonfeld, Mattson, & Riley, 2005; Stresissguth et al., 1996).

In summary, the nature of PAE-related deficits in some cognitive domains have received more attention and are, thus, better characterized in comparison to others, such as

social-cognitive or behavioural problems, which are not well understood. However, the general consensus is that the effects of PAE are severe, permanent and debilitating to both the affected individual and their social network, as well as to the community and the economy. This is particularly true due to its high prevalence.

Fetal Alcohol Spectrum Disorders in South Africa

South Africa presents a unique location for studying FASD as incidence of heavy alcohol consumption during pregnancy is amongst the highest in the world (Croxford & Viljoen, 1999; May et al., 2013). High incidence of maternal drinking occurs in the Cape Coloured population of the Western Cape Province, a population with mixed ancestry (mainly composed of white European settlers, Malaysian slaves, Khoi-San aboriginals, and black Africans). Historically, this population represented the labour force of the wine-producing regions and due to the traditional *dop* system where payment was provided in part in the form of wine (Jacobson, Dodge, Burden, Klorman, & Jacobson, 2011; London, 1999). This remuneration method, despite being outlawed in the 1920s, together with poor social circumstances led to regular and concentrated binge drinking over weekends, as well as heavy alcohol consumption amongst pregnant women. Both persist today in many rural and urban communities, in the Western Cape province, particularly (Jacobson, Jacobson, Molteno, & Odendaal, 2006; May et al., 2000).

Alcohol consumption over weekends has become culturally embedded even in urban communities whose members are no longer actively working in the wine industry. In the Western Cape, over 20% of heavy prenatal alcohol consumption occurs in a binge-like drinking pattern (Croxford & Viljoen, 1999), concentrated over the weekend (Jacobson, Jacobson et al., 2006). This high prevalence of maternal alcohol consumption has led to a high incidence of FAS, as well as other specific diagnoses along the FASD continuum, in the

Western Cape province (Croxford &Viljoen, 1999; Olivier, Urban, Chersich, Temmerma, & Viljoen, 2013). A recent epidemiological study of a rural Western Cape community documented the prevalence of FASD to still be among the highest in the world (May et al., 2013). In this population-based study distribution along the FASD continuum (per 1000) ranged as follows: FAS 59.3 to 91.0; PFAS 45.3 to 69.6; ARND 30.5 to 46.8 resulting in an overall FASD rate of 13.6 to 20.9% (May et al., 2013). Another study, looking exclusively at the prevalence of the most severe cases along the FASD spectrum, found that FAS/PFAS was diagnosed in 17.5% (10% FAS; 7.5% PFAS) of children in a group of rural learners not located in a viticultural region (Olivier et al., 2013). In comparison to the incidence of FAS in the US (0.33-2.2 per 1000; Abel & Sokol, 1991; May & Gossage, 2001), the rates in South Africa are 18-141 times higher (May et al., 2000).

Given that the high prevalence of FASD is not exclusive to viticultural communities, blame can no longer be exclusively allocated to the outlawed *dop* system. Other factors, for example, geographic isolation, as is the case for the towns of Aurora (Western Cape) and De Aar (Northern Cape), rather than viticultural farming, has been identified as a major contributing factor to high incidence of heavy alcohol intake (Olivier et al., 2013). Moreover, South African studies have also shown: (1) a higher FAS/PFAS rate in children born in rural compared to urban areas (May et al., 2000); (2) that binge-drinking (which is related to the high rate of FAS in South Africa) is as high as 20% in rural, 16% in informal and 15% in urban formal communities (Harker et al., 2012); (3) a high mortality rate of mothers of children with FAS/PFAS (May et al., 2000; Olivier et al., 2013). Among these prevalent South African factors, binge drinking may be the most harmful aspect associated not only with FASD but also with accidental injury, domestic violence, unprotected sex and adverse health risks (Naimi et al., 2003; Razvodovsky, 2012). These continuing high incidence rates

of FASD in certain South African communities, and in particular the high rate of FAS cases, makes it clear that heavy prenatal alcohol consumption remains a major public health problem in South Africa.

The high prevalence of FASD, both globally and in South Africa, together with the marked long-term effects of PAE on social-cognitive functioning, warrants further investigation of this domain. Also, considering the limited research, the social deficits seen in children with FASD remains poorly understood. In the following chapter, I present a detailed overview of the existing literature on social-cognitive functioning in FASD. I, furthermore, discuss the mechanisms and processes involved in social cognition within the theoretical frameworks employed in the current research.

CHAPTER TWO: Social cognition and FASD

Introduction

Social communication and relationships are a fundamental part of being human and are related directly to an individual's social development. Social development, in turn, relies on the interplay of an array of social-cognitive factors such as social and emotional cues, the understanding and interpretation thereof, past experiences and the resulting learnt behaviours. Together with an individual's cognitive capacity, these will influence the emergence of social skills and the formation of social relationships, ultimately shaping that individual's social development and ensuing a specific social competence profile.

Social cognition refers to the cognitive processes guiding our social interactions. It is governed by a complex feedback-driven loop of underlying processes that continuously evaluate the reactions, intentions and affective expressions of the self and those of others, which form the social world around us (Adolphs, 2002; Beer & Ochsner, 2006; Fiske & Taylor, 1991). These two components of social cognition, i.e. the perception of the self and the understanding of others, involve numerous stages of processing. Furthermore, the meaning extracted from any social interplay is not static but is instead influenced by the context of the situation in which it occurred (Beer & Ochsner, 2006).

Social cognition is a complex mental and cognitive construct that is framed and taught through social interaction from early infancy. It manifests itself, for example, through the reaction to social stimuli where basic observations are assessed and evaluated against previous experiences of similar situations, and their resultant acquired contextual knowledge (Beer & Ochsner, 2006). Hence, possible outcomes, both inappropriate and appropriate, are internally predicted and evaluated by an individual prior to a behavioural response. Overall,

Social-cognitive skills develop throughout childhood and are shaped by the integration of social interactions and experiences. Therefore, a defective or underdeveloped element within the emerging social cognitive system caused, for example, by an environmental insult such as PAE, may result in impaired in social cognition.

Social Cognition in FASD

As mentioned briefly in the previous chapter (see *Chapter One*, pp. 13-14), PAE affects social-cognitive development, resulting in significant social deficits and poor adaptive behaviours in affected individuals (Carmichael Olsen et al., 1997; O'Connor et al., 2006; Streissguth, Bookstein, Sampson, & Barr, 1993). Descriptions of the social-cognitive behaviour of children with FASD have frequently characterised them as overly friendly and affectionate, as well as outgoing to both familiar persons and strangers, making them vulnerable prone to developing behavioural problems. Streissguth et al. (1996) have argued that social impairment in children with FASD may contribute to the increased rate of delinquency in this population. It has been suggested that such law-breaking behaviour may arise due to a lack of sensitivity and understanding of the rights and feelings of others (Roebuck et al., 1999; Schonfeld et al., 2005). One study showed that children with FASD appear to have poorer social cognition and emotion processing ability compared to children with ADHD and healthy controls (Greenbaum et al., 2009). Specifically, the impairment in social cognition seen in these children with PAE was linked to a range of behavioural problems, whereas alcohol-related deficits in emotion processing were more directly associated with social skills difficulties.

Previous research on the social-cognitive profile of individuals with PAE confirmed poorer social skills in children with PAE compared to healthy controls. Additionally, this

impairment in social cognition appears to be independent of overall cognitive functioning (Thomas, Kelly, Mattson, & Riley, 1998; Whaley, O'Connor, & Gunderson, 2001). Moreover, such deficits are characterised by an arrested rather than delayed developmental state, and seem to persist into adulthood (Carmichael-Olson, Morse, & Huffine, 1998; Streissguth & O'Malley, 2000; Thomas et al., 1998).

Theoretical Frameworks

During the development of social skills, emotions play an essential and primary role in providing feedback following social encounters and in promoting social interactions (Halberstadt, Denham, & Dunsmore, 2001). In functionalist theories of emotion, emotions are considered important components of behaviour because they motivate and facilitate the interpretation of specific situations. Within that framework, they have a vital interpsychological function (Ekman, 1984, 1993; Plutchick, 1980). In this context, another individual's emotions provide information about their behaviours and their mental states. Furthermore, emotions are considered to serve an internal organizational and motivational function guiding variable goal-directed behaviours (Malatesta, 1990; Saarni, Mumme, & Campos, 1998). In this regard, emotions are considered to play an adaptive role in our behaviour. This notion is supported by neurophysiology research, which shows that complex neural interconnections exist between cortical areas related to emotion and those of cognitive functioning (Kandel & Kupferman, 1995). Hence, aside from the function our own emotions serve in shaping our social experiences, reading and correctly understanding the emotions displayed by others is crucial in eliciting a specific behavioural response.

Social information processing. As a response to interacting with our peers, the recognition and interpretation of other's emotions is critical to effective social interactions

(Halberstadt et al., 2001). Over the past three decades, researchers have developed and modified various social information processing (SIP) models (Crick & Dodge, 1994; Dodge, 1985, 1986; Dodge & Crick, 1990; Ladd & Crick, 1989; Slaby & Guerra, 1988), in attempting to explain children's social behaviour and adjustment. The basic concept underlying SIP models is that children's behaviour and reactions towards specific social situations are based on their understanding and interpretation of the situation (Piaget 1932/1965; Turiel, 1998). SIP entails numerous sequential steps beginning, for example, with a child (1) attending to a situation; (2) encoding and interpreting the social cues; and (3) responding to the situation in a very specific manner influenced by a multitude of highly subjective factors and experiences. Crick and Dodge (1994) developed a detailed model demonstrating how children are said to arrive at a certain reaction to a specific situation.

The basis of this model are the social-cognitive processes observed in typically developing children during peer interactions, which include receiving and interpreting social cues, which in turn result in a reaction to the social environment. One of the most crucial social signals serving to reveal other's intentions, are their facial emotional expressions. Additionally, emotional display is also the manner by which individuals transmit an affective message (Izard, Schultz, Fine, Youngstrom, & Ackerman, 1999/2000). Expressions of facial emotions thus play a critical role in social interactions as they provide clues about a person's internal states, or the state that person wishes to convey. Problematically, the initial SIP model did not include emotion as an influencing element. However, the Crick and Dodge SIP model has since been modified to include emotion processes, such as emotion production, emotion regulation or emotion recognition, at various steps throughout the model, which may indeed influence the outcome of a given social situation (Lemerise & Arsenio, 2000). Given

that emotions impact our behaviour and thus ultimately influence our social interactions, the importance of including emotion within an integrated SIP model now seems indisputable.

The modified Crick and Dodge SIP model (see Figure 2.1; Lemerise & Arsenio, 2000) divides the process of children's social adjustment into six major steps. In addition, the modified model takes into consideration the motivational and adaptive role emotions play at every step of social information processing. The behaviour or reaction to a certain social situation is, therefore, highly dependent on the intact functioning of emotion processing throughout the model. One may, thus, hypothesize that a breakdown or deficit in emotion processing at any step of the social information cycle (e.g., a deficit in interpreting one's own emotion or that of others) will result in an inappropriate reaction to another's behaviour, based on a false interpretation of that person's behaviour. The model demonstrates how numerous cognitive, subjective and environmental factors need to work in unison, and together with an individual's internal and external emotion projection and comprehension, before a specific behavioural outcome can be elicited. The model relies on the interplay of an individual's personal experiences and an intact network to generate a multitude of favourable and unfavourable reactions to a specific situation. Hence, a deficit in an individual's emotional processing or in their understanding of other's mental states may, thus, be the underlying cause of an unsuitable or socially inappropriate reaction.

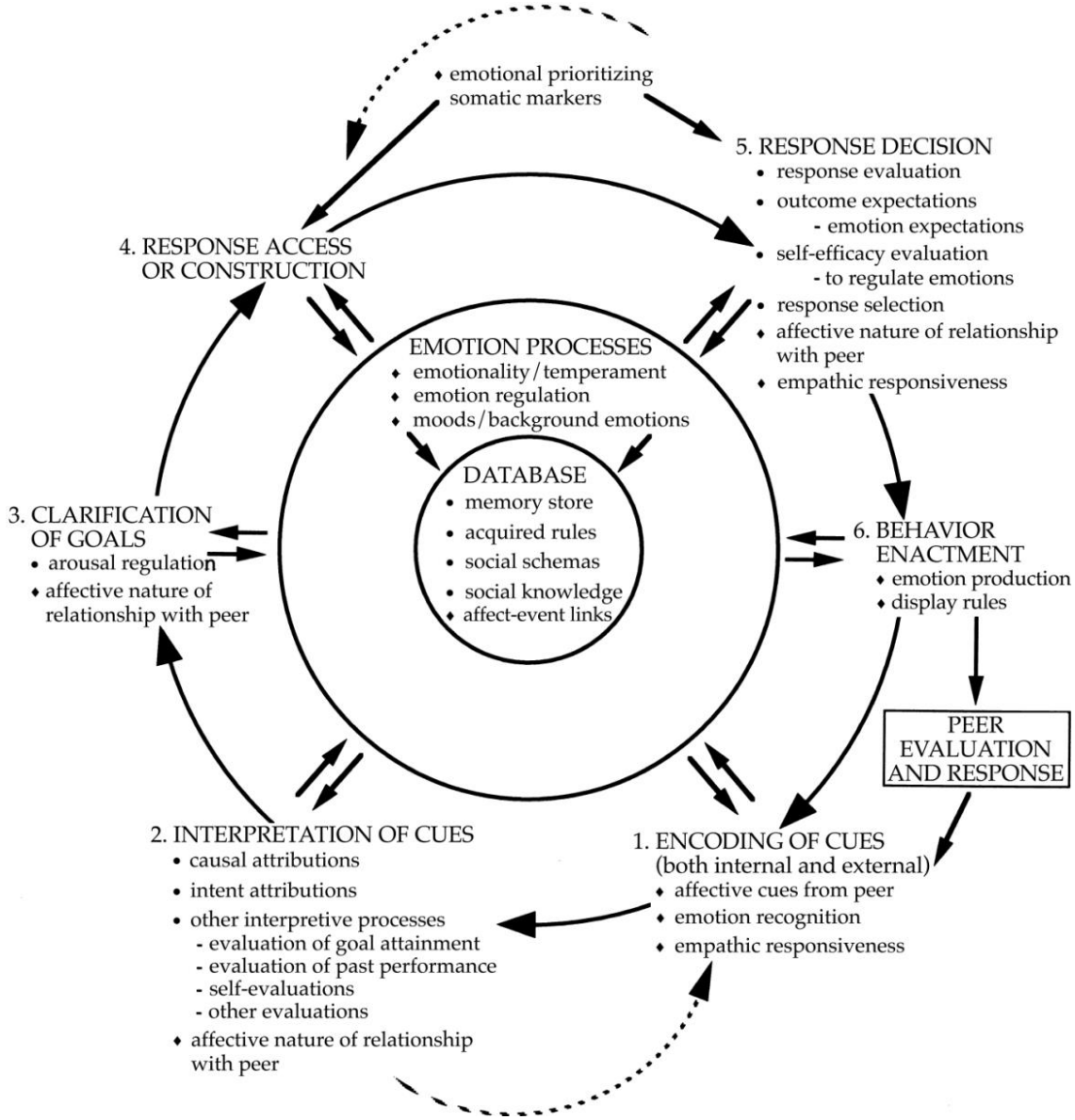

Figure 2.1 The modified social information processing model integrating of emotion processes and cognition. Note: From "E. A. Lemerise & W. F. Arsenio (2000). An Integrated Model of Emotion Processes and Cognition in Social Information Processing Child Development, 71, 107-118."

Affective social competence. Social-cognitive abilities emerge from mental and cognitive processes and are considered an integral part of the concept of social competence. Generally, the very broad term of social competence has been regarded as a central component of social interactions. One attempt at providing some level of specification as to which processes are encompassed by the term social competence has conceptualised it as incorporating social skills ability, peer relations and their functionality, as well as the understanding of social reactions (Rose-Krasnor, 1997).

Halberstadt and colleagues (2001) have devised a multifaceted model of affective social competence (ASC; see Figure 2.2). They incorporating a range of factors in an attempt to tease apart the many integrative skills said to be required for social interactions. They emphasized that emotions, both one's own and those of others, make up the key factors that guide our social relations. In their definition of ASC, they delineate it as (1) the successful communication of one's own and others' affect; (2) the interpretation and response thereto; and (3) the awareness, acceptance and ability to regulate one's own emotions. As a result, the model is organised around three core aspects of affect: sending, receiving and experiencing. Within these three aspects, the model outlines four progressive abilities that are crucial for successful social interactions. These are the (1) awareness and (2) identification of affective messages; (3) ability to apply the affective message within the current social situation; and (4) management of the affective message, which includes the ability to appropriately regulate affect depending on what the context requires.

In summary, the current model describes affective social competence as a complex process that involves the hierarchical development of a range of integral affective skills that are governed by past experiences and ever-changing social encounters. Furthermore, these

processes are influenced by a variety of individual differences that moderate the successful manifestation of ASC, which in turn govern an individual's social interactions.

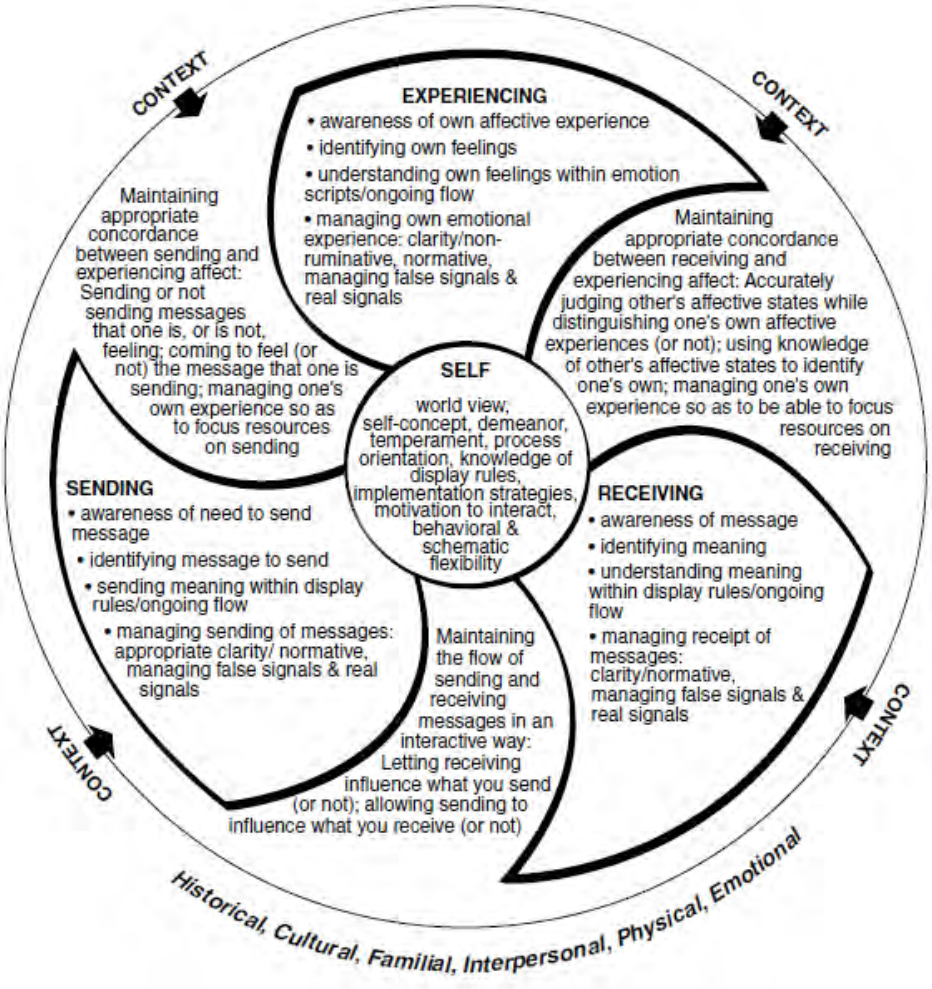

Figure 2.2
Depiction of the affective social competence model. Note: From "A. G. Halberstadt, S. A. Denham & J.C. Dunsmore (2001). Affective Social Competence, Social Development, 10, 1, 79-117."

Relevance of processing models to FASD. Both the SIP and ASC models suggest that either the successful or impaired implementation of previously acquired skills at any level within the model will ultimately affect the development of subsequent abilities. In other words, individuals who have difficulties in the early steps of social information or affective

social processing will also have difficulties with processing at later stages of the model due to the hierarchical progression and interdependence of these abilities. For example, trouble with encoding (SIP model) or receiving (ASC model) social cues could arise due to deficits in facial emotion processing, which in turn could be the facilitator of social-cognitive deficits.

The modified SIP model highlights the important processes at each level and how these build on each other to form an intricate and multifaceted system that guides the development of social understanding and behaviour. Interrupted or incorrect processing at any step may, therefore, ultimately influence the further development of acquired knowledge and behaviour in subsequent levels of the model. Furthermore, perhaps the earlier the processing chain is disrupted the more severe the resulting deficits in social cognition. For example, if the affective cues received from peers are misread or the emotions portrayed are not recognised correctly at the first level, the subsequent steps, e.g., the interpretation of the cues, will be flawed too, resulting in an inappropriate response.

Similarly, in the ASC model, the successful integration of social communication and social skills acquisition can break down within any component of the related abilities. For example, if within the receiving component, abilities such as identifying the meaning of the received affect are impaired, the management of sending and receiving affective messages is disrupted. Also, as a result, maintaining the concordance between receiving an affective message and successfully experiencing that affect will also be affected.

In an attempt to understand the social behavioural problems evident in individuals with FASD, one might investigate these individuals' competence at the various levels of both the integrated emotion SIP and ASC models. Here, specific PAE-related deficits at each step within a model may be identified as the underlying mechanisms contributing to the atypical

social response of children with FASD. No previous study has used the ASC model to investigate social cognition in FASD. By contrast, support for using the SIP model in their field comes from findings suggesting that children with FASD do indeed show impairment in social information processing (Coggins, 1997; Howell et al., 2006). For example, social problem solving skills and cognitive and executive impairments affecting these skills have been examined in adolescents (13-18 years) with heavy PAE, with data interpreted following the framework of Crick and Dodge's (1994) SIP model (McGee, Bjorkquist, Price, Mattson, & Riley, 2009). In that study, children with PAE demonstrated impaired ability at various steps of the SIP model when viewing video vignettes of provocative situations: clarification of goals, response generation and evaluation. Participants also showed impaired social problem-solving skills based on the administration of a self-report measure (Social Problem Solving Inventory, Revised; SPSI-R). Furthermore, participants also had deficits on a range of executive functioning measures. These impairments were consistent with the steps delineated in the SIP model and were thought likely to lead to problems with social functioning.

A more recent study looked at only one aspect of the Crick and Dodge SIP model, response generation (Stevens et al., 2012). It examined, using the Children's Interpersonal Problem Solving questionnaire (ChIPS; Shure & Spivack, 1985), how children (aged 9-16) with FASD process social information. In this study, children with FASD were able to generate fewer solutions to the social dilemmas presented but the ChIPS compared to healthy control participants. The authors suggested that this impaired social problem solving ability and the failure to implement an appropriate strategy may be responsible, in part, for social and behaviour problems generally seen in children with FASD.

When the current research project was designed (in early 2011) only the two studies (reviewed above) had examined social-cognitive abilities in children with FASD by examining aspects of Crick and Dodge's (1994) SIP model. None had yet considered including the impact of emotion processing, which is incorporated in the modified SIP model (Lemerise & Arsenio, 2000). Furthermore, a literature search yielded no previous research that included an investigation of social cognition including affective social competence in FASD. Since completion of data collection for the current research, a recent publication has evaluated emotion processing in FASD within the modified SIP framework (Kerns, Siklos, Baker, & Müller, 2016). Kerns and colleagues investigated emotion recognition in 8- to 14-year old children with a FASD diagnosis by measuring performance on tasks recognizing facial expressions, emotional tone of voice, and body language. Facial expression and emotion recognition was assessed with established tests, which used photographs of faces displaying different affects. Children with FASD had difficulty recognizing emotions displayed on adult but not child faces compared to healthy, age- and sex- matched control participants. The lack of between-group differences on child stimuli was suggested to be related to the simplicity of deciphering the specific task stimuli. Limitations of this study, as listed by the authors, include a small sample size, challenges of recruiting FASD participants and well-matched controls, possible effects of impairments in attention in these individuals, and other psycho-emotional factors that may have influenced performance due the nature of specific tasks.

Importantly, as suggested above, while it has been established that social impairment is a major and debilitating consequence of PAE, very little is known about the underlying mechanisms of these social-cognitive deficits. Some consider them to be secondary to other

cognitive and executive function impairments, whereas others have suggested a possible deficit in the social-cognitive domain of Theory of Mind (ToM), the ability to infer the mental states of others, as the underlying mechanism (Coggins, Olswang, Olson, & Timler, 2003; Thomas et al., 1998). Thus, deficits in ToM may contribute to difficulties in interpreting behaviour of others and correctly identifying affective cues and recognising emotions.

In the literature in general, emotion processing and ToM have been the two mental constructs frequently utilised to study social cognition (Green, Olivier, Crawley, Penn, & Silverstein, 2005; Penn, Addington, & Pinkham, 2006). A functional relation between these two processes seems evident in the models reviewed above. An important consideration regarding the association between emotion processing and ToM is the fact that to determine someone else's intentions (i.e., ToM) one needs to assess their emotional status (i.e., emotion processing), which suggests that these processes are interdependent (Ochsner, 2008). Therefore, one aim of the current research project was to include a comprehensive developmental battery of ToM measures, including tasks tapping into emotion recognition, in an attempt to identify steps in the modified SIP and ACS models that may be impaired due to deficits in ToM.

Summary of previous ToM and FASD literature. Previous research within the FASD literature has provided some initial insight into ToM abilities in individuals with PAE. One of these studies investigated ToM in young children (aged 4-8 years) with FASD in relation to executive function and age (Rasmussen, Wyper, & Talwar, 2009). The findings suggested that children with FASD have difficulties in ToM; 44% of them failed one or both of the ToM tasks administered, compared to only 25% of the healthy controls. Furthermore, and consistent with previous findings (Thomas et al., 1998; Whaley et al., 2001) suggesting that

social deficits become more pronounced with age, Rasmussen and colleagues found that ToM performance was worse among the older children in their cohort. The authors concluded that among children with FASD, ToM deficits become more pronounced with age and persist into adulthood.

Another study examined social cognition by administering a social-cognitive battery (developed by Salzman-Benaiah & Lalonde, 2007) containing ToM-related tasks dealing with False Belief, intention, deception, and interpretive ToM (Greenbaum et al., 2009). Social cognition was further investigated by assessing emotion processing ability using the Minnesota Test of Affective Processing (MNTAP). This study found that children (age 6-13 years) with FASD have poorer social cognition and facial emotion processing ability than children with attention deficit hyperactivity disorder (ADHD) and normal controls. Similarly, parent and teachers' reports showed more behavioural problems and poorer social skills in children with FASD and ADHD compared to typically developing children in the same study.

A fairly recent study (Rasmussen et al., 2013) examined neuropsychological impairments in children (age 6-16 years) with FASD on the NEPSY-II (a Developmental Neuropsychological Assessment–Second edition; Korkman, Kirk, & Kemp, 2007). Children with FASD showed no impairments on the ToM subtest of the NEPSY-II. However, the authors suggested that this may be due to the NEPSY-II ToM subtest's assessing too broad a spectrum of ToM abilities.

Limitations of Previous Research

Research with clinical populations has it its own unique challenges. Most studies investigating FASD recruit participants through support centres or clinics and on the basis of an FASD diagnosis. In other words, participants are recruited retrospectively, and thus

important information pertaining to the pre-and postnatal environment is not available. In addition to not having access to concurrent alcohol exposure measures, other variables, such as maternal drug use during pregnancy, cannot be controlled for as potential confounders of the effect of alcohol. In addition, the cognitive deficits in children across the different subtypes of FASD are related to the degree of alcohol exposure. Thus, studies in which exposed and unexposed children are examined include many who do not have the same cognitive profile. It is also often difficult and costly to prospectively recruit a large number of FASD participants with individuals of all subtypes represented equally. At the same time, it is challenging to recruit a suitable control group against whom the alcohol-exposed participants can be compared.

The previous studies that examined social cognition in FASD within the SIP framework faced these problems and acknowledged them as limitations. For example, Mc Gee and colleagues (2009) compared a group of children with PAE, in the absence of a FASD diagnosis, to controls. Their findings may, thus, not be representative of the full range of the FASD population. Furthermore, in their group analyses, Stevens et al. (2012) treated the FASD group as one diagnostic group, without differentiating between participants from the various subtypes along the spectrum. Lastly, a limited sample size is another critique of previous social-cognitive FASD research and noted, for example, by Kerns and colleagues (2016).

The Current Research Project's Approach

In the context of the current research project, it is important to recognize that humans are exceptionally social beings. Adults and children constantly observe the behaviours and reactions of others in an attempt to figure out what they are thinking and feeling. The ability

to make social inferences to predict the behaviour of others contributes to the ability to understand social situations, and facilitates building and sustaining peer relationships. Hence, the mentalizing process of ToM is considered the most crucial component in early childhood development (Flavell & Miller, 1998; Harris, 2006), and the most important developmental milestone for social cognition (Astington, 1993; Astington & Dack, 2008). Difficulty in interacting appropriately with one's peers and poor social skills development in children with PAE could, therefore, indeed be indicative of deficits in ToM, including the attribution of affective states.

Although children with FASD are known to have deficits in social-cognitive functioning (Coggins, 1997; Jacobson & Jacobson, 1999; Jacobson et al., 2004; Kodituwakku, 2007), and precursors of affective problems are already seen in infancy (Hay et al., 2004; Molteno, Jacobson, Carter, Dodge & Jacobson, 2014; O'Connor, Sigman, & Brill, 1987), very little is known about mechanisms leading to the socioemotional deficits of FASD. The inability to maintain successful and effective social relationships puts an individual at risk for an array of mental and physical health issues, both well established in children affected by PAE. Hence, the aim of the current research project is to investigate potential deficits within aspects of social information processing and affective social competence, which may be responsible for the social-cognitive deficits seen in children with FASD. It is, however, crucial not to simply explain how an individual's impairment may cause a socially inappropriate reaction; it is also important to provide an explanation regarding the source of the disability. The current research project, therefore, aimed at providing insight pertaining to 'where' and also 'how' the social information/emotion processing pathway or the sequence and integration of affective social competence are interrupted as a consequence of PAE.

This study takes as its guiding principle the following: When examining these social-cognitive constructs, it is necessary to first consider how ToM and emotion recognition fit into the models. The formation of suitable social responses begins at the most basic levels: encoding of cues, correctly recognising emotions, and interpreting such cues, by understanding the meaning and intent of the portrayed emotions. Thus, these variables appear to impact the progression of the SIP model at a very early and basic level. Correctly identifying emotions is embedded in the ability to recognize affect, a necessary component at level 1 of the model. The mechanisms underlying ToM in general (i.e., the ability to infer the mental state of others) appear to occur at level 2 of the model. Here, 'intent attributions' and the 'evaluation of other' are attributable to fundamental ToM processes. This trajectory suggests that with impaired ToM ability the social information process would break down at these early levels and, thus, follow a flawed path throughout the remaining model. Furthermore, the 'database' of the model, upon which other levels rely, also appears to be related to ToM ability, in particular with regards to 'social schemas' and 'social knowledge'.

As previously indicated, affect recognition seems to play a vital role at the very first level of SIP, when the social cues are encoded. An incorrect interpretation at this very early level due to impaired affect recognition ability may, therefore, finally determine an inappropriate response. The problem arises at the next step (level 2), when the emotions displayed by a peer need to be interpreted. If the emotions are not recognised correctly (level 1), they will ultimately not be interpreted correctly (level 2), which will consequently result in an incorrect progression of the model and resulting inappropriate social behaviour.

Similarly, in the ASC model both emotion recognition and ToM ability appear crucial for the second skill area, namely receiving affective messages. The ability to recognise

emotions provides an individual with feedback about his/her own but also about another person's intentions. This step of the ASC model requires not only the awareness that an affective message was sent, but also the ability to correctly recognize and interpret the affect given the current situation. In other words, an individual needs to recognize the affect displayed (i.e., process the emotion) and then also understand and interpret what the other person is thinking/feeling given the affect they are displaying (i.e., ToM ability).

The existing connection between ToM and emotion processing supports the current project's approach. Firstly, social-cognitive impairment is investigated by evaluating ToM ability and secondly, emotion processing is examined, both in the context of the modified SIP and ASC models reviewed above. The rationale for employing this methodology was to identify ToM and emotion processing related processes within the specific steps of the SIP/ASC models that may be affected by PAE, and which in turn might result in maladaptive social behaviours in the FASD population. Therefore, the first goal was to establish whether impairments in ToM ability exist, which are potentially responsible for flawed cognitive interpretations of social cues, which in turn result in inappropriate reactions to social stimuli. A more basic skill, which is considered a prerequisite to the higher-order interpretation processes involved in ToM, is emotion processing. Thus, as a second examination emotion processing was explored, focusing specifically on facial emotion recognition. This investigation was aimed at establishing whether the potential deficits in ToM are a direct consequence of the effects of PAE, or are perhaps instead rooted in an impairment at the more basic emotion processing step, a precursor to ToM.

The current social-cognitive FASD research project is the first to recruit its participants, both alcohol exposed and controls, prospectively during pregnancy. This

approach enabled me to collect detailed and concurrent pre- and postnatal information. This design also made it possible to compare performance based on FASD diagnosis and on continuous measures of alcohol exposure that do not consider the diagnosis of the individual. Thus, impairments in ToM or emotion processing can be directly related to severity of PAE independent of FASD diagnosis.

Considering that ToM and emotion processing can be independently impaired and unrelated to any other cognitive deficits which may be present, children of the different subtypes of FASD may also display different patterns or degrees of impairment. Hence, in the current research project performance on administered tasks was compared across all diagnostic groups and in comparison to suitable control participants recruited from the same community. This approach allows for the characterisation of skills in relation to severity of alcohol exposure as it can demonstrate each diagnostic group's combination of skills and impairments across the spectrum.

Given the dearth of literature on FASD and the mechanisms of social-cognitive impairments, the current research aimed initially to evaluate the degree to which ToM is impaired. Based on that investigation, the research continued to explore potential impairments in emotion recognition and beyond that using neuroimaging to explore potential differences in neuronal activation during the processes of emotion recognition. Throughout, the research aimed to overcome the limitations of previous research in these domains by (1) assessing FASD and control participants that were recruited prospectively from the same community; (2) administering a novel test battery considering the social-cognitive processes embedded in a SIP and ASC model and (3) evaluating group differences in performance separately across the diagnostic groups and in comparison to suitable controls as well as considering the effect

of continuous measures of PAE independent of FASD diagnosis. Furthermore, emotion recognition and social information processing abilities have not previously been found to be related to intellectual functioning (Baum & Nowicki, 1998; Gouze, 1987; Nowicki & Duke, 1994). However, in order to control for the potential mediating effect of general intelligence and EF on the effect of alcohol, the current research evaluated all significant results in relation to these variables.

In summary, in this chapter, a review of the previous social-cognitive literature relating to PAE and its strengths and limitations was discussed to provide insight into the rationale underlying the theoretical approach of the current research project. I presented the theoretical concepts that were employed, which provided the framework for the social-cognitive concepts that were explored. These concepts were discussed to elucidate the new approach taken to examine the social-cognitive impairments in children with FASD as well as highlighting the strengths and novelty of this research. The next chapter provides more details on the current research project, particularly in regard to its general research implementation and methodology.

CHAPTER THREE: General methods and research context

Introduction

Evidence reviewed in the previous two chapters allows the conclusion that children with FASD do indeed have impairments in social cognition. Moreover, these impairments may be related specifically to ToM and the processing of affective expressions. Considering the real life social and behavioural problems that can stem from impairments in social cognition, this topic requires further detailed investigation. Social cognition is not an isolated or independent cognitive domain but should be regarded as a complex and integrated system impacted by various cognitive processes. Social or behavioural problems may, however, also be secondary to other underlying cognitive deficits, deficits nested in domains other than social cognition, which children with FASD are known to have. Thus, both primary and secondary deficits need be considered and appropriate measures need to be controlled for when investigating elements of social cognition. Acknowledging social cognition as a multifaceted system requires development of methods that can measure deficits within that system. The aim of the current research project was to investigate social cognition in children with FASD by exploring their abilities and impairments within the domain of social information processing and affective social competence. This aim was achieved by investigating (1) ToM and (2) emotion recognition, as potential determinants of deficits in social cognition, which are considered fundamental concepts necessary for successful social interaction.

This research project was aimed at identifying specific socioemotional deficits FASD children may have within the domain of social information processing and affective social competence. As part of this investigation, I administered individual cognitive tasks to establish which specific domains (e.g., general intellectual ability, EF, etc.) and abilities (e.g.,

WM, affect recognition, affective appraisal, etc.) are impaired and which are intact, and which are more and less affected by heavy PAE. For this purpose, I made comparisons across the different FASD subgroups allowing me to characterise potential deficits by (1) determining whether increased PAE is related to a greater number and severity of deficits; or (2) whether specific subgroups along the spectrum of FASD remain unaffected or are affected differently on ToM or emotion processing. To identify and understand the mechanisms underlying impaired behaviours, I considered any deficits I found in relation to other potential cognitive impairments.

Research Plan and Setting

The current research is comprised of three studies aimed at exploring the nature of the social-cognitive deficits in children with FASD, which were assessed both behaviourally and by means of neuroimaging. Study I was an exploratory study that investigated a broad range of ToM abilities. Based on these findings, Study II was then designed to further explore the domains found to be significantly impacted and most strongly related to the effects of PAE. Hence, Study II was designed to further investigate the ability to read people's facial emotions, which presumably are used to make social judgments about their behaviour and feelings based on the affective social cues provided. For this purpose, affective appraisal ability, i.e. the ability to correctly identify basic facial emotions, was examined in children with FASD. WM is an important general cognitive gatekeeper and has previously been shown to be impaired in individuals with FASD. Thus, affective appraisal was assessed in consideration of the participant's WM abilities, given that due to its design, performance on the affective appraisal task may be confounded by WM deficits. Concurrently, Study II served as a pilot study for Study III, in which affective appraisal was examined using neuroimaging.

Study III explored potential differences in cortico-limbic activation in children with FASD during an affective appraisal task using functional magnetic resonance imaging (fMRI).

The high incidence of FASD makes the Western Cape an ideal setting to prospectively recruit a large number of participants during the prenatal phase (Jacobson et al., 2011). A prospective recruitment approach provides a rich documentation of the history of PAE for each individual because the information is collected concurrently when the behaviour is ongoing and current. In contrast to the retrospective recruitment methods, this approach allows for the generation of continuous alcohol measures, which can be used to further study the effect of PAE in these individuals independent of their respective FASD diagnosis. Much of the behavioural and neuroimaging research on the neurodevelopmental sequelae of prenatal alcohol exposure consists of case-control studies, in which children and adolescents diagnosed with FASD are compared with typically developing, community-matched controls (e.g., Fryer et al., 2007; Mattson et al., 1997; Sowell et al., 2001, 2002, 2007). Because no contemporaneous report of maternal alcohol consumption during pregnancy is available, these studies do not provide the data needed to examine the relations between specific doses and patterns of PAE, and developmental outcome. Although four major prospective longitudinal PAE cohort studies have been conducted in Seattle, Atlanta, Pittsburgh, and Detroit, in which mothers were interviewed, those studies did not examine behavioural and neuroimaging outcomes in relation to FASD diagnoses (Coles et al., 1997; Day et al., 2002; Jacobson et al., 2004; Streissguth et al., 1994).

Methods

Participants

Participants for the current social-cognitive research project were recruited from an established longitudinal cohort in Cape Town from the Child Development Research Laboratory (CDRL) at the University of Cape Town. Thus, the methodological background, pertaining in particular to participant recruitment and generation of certain research measures, is similar for all three studies of this doctoral dissertation. To avoid redundant repetition I present the key background information common to Study I, II and III below.

Recruitment of the current longitudinal cohort. From 1999 to 2002, 227 pregnant women were recruited through an antenatal clinic, which was selected based due to the high incidence of heavy prenatal alcohol consumption (Croxford & Viljoen, 1999; Jacobson et al., 2008). Infants born to these women were enrolled in the Cape Town longitudinal cohort study. Because recruitment occurred during pregnancy, extensive information regarding maternal alcohol consumption could be obtained using a timeline follow-back approach (Jacobson et al., 2008; Sokol, Martier, & Ernhart, 1983). Each pregnant woman was interviewed twice regarding the incidence and amount of day-to-day alcohol consumption during a typical 2-week period.

Units of alcohol. The volume of each type of alcoholic beverage consumed each day was converted to absolute alcohol (AA), a measure of the volume of pure alcohol consumed. One ounce of AA is the equivalent of 2 standard drinks (a specified amount of pure alcohol; Jacobson et al., 2002). At the time that the cohort was originally recruited, an incident of binge drinking was defined as 5 standard drinks per session for men and women. Any women who reported drinking at least 14 standard drinks a week (i.e., 1.0 oz AA/day) or had engaged

in binge drinking (5 drinks/occasion) at least twice per month during the first trimester of pregnancy were invited to enrol in the study. These women were considered to be heavy drinkers, in contrast to moderate drinkers (alcohol exposure of 0.5 to 0.99 oz AA/day) and light drinkers (alcohol exposure of < 0.5 oz AA/day). NIAAA has since redefined binge drinking for women as 4 drinks per occasion. In the current research, the newer definition for binge drinking was applied in the binge analyses.

Women from the same antenatal clinic who were within 2 weeks of gestation of a recruited heavy drinking woman were also invited to participate in the study if they reported abstaining or only drinking minimally (i.e., <7 standard drinks per week) and did not binge drink.

Dysmorphology exam and FASD diagnosis. The children in the cohort were examined to obtain a FASD diagnosis using the revised IOM criteria (Hoyme et al., 2005). During a clinic conducted in 2005, each child was independently examined for growth and FAS anomalies using a standard protocol by two expert dysmorphologists (HE Hoyme, MD; LK Robinson, MD). There was substantial agreement between the two U.S.-based dysmorphologists on their assessments of all dysmorphic features, particularly palpebral fissure length and philtrum and vermilion ratings based on the Astley and Clarren (2001) rating scales (*r*-values = .80, .84, and .77, respectively). There was also substantial agreement between them and the Cape Town-based dysmorphologist (NK; median *r* = .78), who evaluated those children who could not be scheduled for the clinic.

FAS and PFAS diagnoses were subsequently determined at case conferences by the two U.S.-based dysmorphologists (HEH & LKR) and the two primary investigators of the longitudinal Cape Town study (Profs. Sandra Jacobson & Joseph Jacobson). Diagnoses were made using data from the clinical examination together with the prenatal alcohol history

obtained during the prospective interviews conducted during pregnancy. Subsequent FASD diagnostic clinics have been held in 2009 and 2013, which have confirmed these diagnoses.

The social-cognitive cohort. In the cohort, among the children of heavy drinking mothers (i.e., excluding controls), 19.5% of were diagnosed with FAS and 26.8% met the criteria for PFAS, while 53.7% were born to heavy drinkers who did not exhibit the FAS facial anomalies. The latter group thus comprised a heavily exposed non-syndromal group (HE). Individuals who make up this category may lack the distinctive alcohol-related facial features and growth retardation (Suttie et al., 2013). However, even though these individuals may not display the requisite syndromal physical anomalies, many, if not most, may demonstrate significant neurobehavioural and cognitive deficits related to a confirmed history of heavy prenatal alcohol exposure (Crocker, Vaurio, Riley, & Mattson, 2011; Jacobson et al., 2004).

The social-cognitive cohort was divided into four diagnostic groups: FAS, PFAS, HE non-syndromal, and non- or minimally exposed control groups. The majority (81.7%) of the heavy drinking women in the cohort binge drank (4 or more drinks/occasion). On average, the heavy drinking mothers drank 7.04 drinks/occasion 1-2 days/week. All but one of the women in the control group abstained; 1 woman drank 2-3 drinks on less than 3 occasions during her pregnancy. Thus, as has been noted anecdotally, the drinking pattern in the Western Cape is bimodal: women either abstain or drink minimally or else they tend to concentrate their alcohol use on weekends and binge drink (Jacobson, Jacobson, Stanton, Meintjes & Molteno, 2011). Participants for each of the three studies presented in this dissertation were recruited from the social-cognitive cohort described above.

Measures

Aside from domain-specific research tasks (each of which is covered separately in each study description in the chapters to follow), a range of cognitive and behavioural measures were administered across all three studies. These measures were, primarily, administered as part of the neuropsychological test battery used in Study I. The IQ and EF data collected during Study I were, however, also used for comparative analyses in the other two studies. To avoid repetition in the subsequent chapters, below I provide an overview of these measures and refer to this section for further details in the write-up of each study.

Currently, culturally appropriate and unbiased tests are not available for many cognitive domains assessed within neuropsychological evaluations in South Africa, both in clinical and research settings (Foxcroft, Paterson, Le Roux, & Herbst, 2004). For this reason, in the current research project, and as is common practice in South African-based cognitive research, age-appropriate Western measures were utilised. However, Western norms are not suitable for comparison when working with a socioeconomically disadvantaged, heterogeneous paediatric South African population. Therefore, to evaluate the outcomes on the standardised tests administered, performance of FASD subgroups on each task was evaluated against the control group's performance on the same task.

Moreover, it is well documented that language is one of the most important single moderators affecting performance on psychometric measures (Nell, 1994). In order to adjust for a multi-cultural and multi-lingual (English and Afrikaans speaking participants) research setting, the measures described below were adapted for the South African context and translated into Afrikaans by native speakers and back-translated into English. During this process great caution was taken (1) not to change the measures' original meanings and (2) to keep the items at the same level of complexity. Also, for instances, where English items were

deemed culturally unsuitable for English-speakers in the cohort, these were replaced with an equivalent item more suitable for the South African context. For non-verbal items, such as the WISC-IV working memory and processing speed subtest, the instructions were simply translated and the stimuli remained unchanged. The tests that were specifically translated and adapted especially for the current study (i.e., instructions for the EF tasks, NEPSY-II ToM subtest and the ToM battery) were translated and then checked by fluent bilinguals to ensure that the meaning was exactly the same.

General intellectual functioning (IQ). The WISC-IV (Wechsler, 2003) was administered to assess general intellectual ability. These data were used to determine whether effects of alcohol exposure on the social-cognitive endpoints studied here were domain-specific or reflected general intellectual deficits. The WISC-IV is well-established and the most frequently used standardized assessment of childhood intelligence (Watkins, Wilson, Kotze, & Carbone, 2006) with extensive validity and reliability evidence (e.g., Prifitera, Saklofske, & Weiss, 2005). The full WISC, which includes the following subtests was administered: Block Design, Similarities, Picture Completion, Digit Span, Coding, Vocabulary, Matrix Reasoning, Comprehension, Arithmetic and Symbol Search.

The WISC-IV was translated into Afrikaans by a clinical psychologist whose first language is Afrikaans. The WISC-IV was, thus, administered in either English or Afrikaans, depending on the child's schooling or language proficiency.

At the CDRL, WISC IQ scores have previously been estimated from these subtests using Sattler's (1992) formula for computing Short Form IQ; validity coefficients for Sattler Short Form IQ based on five or more subtests consistently exceeded $r = .90$. In a 5-year follow-up assessment of the children from the longitudinal cohort, the Junior South African Intelligence Scale (JSAIS; Madge, van den Berg, Robinson & Landman, 1981), which is

available in Afrikaans and English was administered. The J-SAIS has been normed for South African children in both English and Afrikaans. Sixty-two of the children from the 5-year follow-up also took part in Study I of this dissertation, which was part of their 9-year follow-up assessment. During this assessment, the WISC-IV was administered. IQ scores obtained using the JSAIS at 5 years were strongly correlated with the Study I WISC scores, $r = .77$, $p <$ 001. These data, thus, provide validation for use of this version of the WISC in research.

Executive function (EF). EF has been conceptualised as multiple processes that are interrelated and inter-dependent and work together to enable cognitive executive control (Anderson, 2002). Following the evidence documenting EF impairments in children with PAE, it was necessary to assess whether the effects of fetal alcohol exposure on ToM and on emotion recognition are domain-specific or reflect alcohol-related deficits in EF. Thus, a range of EF tasks were chosen based on their ability to assess a participant's performance across several inter-related EF domains. In his review of EF (2002), Anderson proposes a model consisting of four related executive systems, namely 'attentional control', 'information processing', 'goal setting' and 'cognitive flexibility'. Each of these systems or domains is comprised of several EF processes or abilities. In the current research, multiple standardised neuropsychological tests were administered to assess these EFs. However, based on EF deficits shown to be common in individuals with PAE, EF measures were selected to specifically control for alcohol-related impairments in EF and, thus, adaptations were made to the domains proposed by Anderson's model (2002). Hence, based on evidence that WM is an attentional domain most affected by PAE (Burden et al., 2005; Connor et al., 2000), and given the nature of the other cognitive tasks to be administered (see *Measures* section in *Chapter Five*, pp. 103-107), I decided to examine WM as a separate domain, and not as an ability embedded in the 'cognitive flexibility' domain as suggested by Anderson (2002).

In addition, goal setting, attentional control, and cognitive flexibility were assessed. Goal setting was chosen because according to Anderson's model (2002) this domain includes the ability to plan and requires strategic organisation and children with PAE have been shown to have deficits in planning compared to healthy controls (Kodituwakku, et al., 1995; Mattson et al., 1999). Similarly, attentional control was chosen because it includes inhibitory control (Anderson, 2002) and alcohol-exposed children have been shown to have poor response inhibition (Kodituwakku, et al., 1995; Mattson et al., 1999).

Working memory. *Digit Span backwards* is the second part of the WISC-IV digit span subtest and was analysed separately as a measure of working memory in the measurement of EF. This task has been shown to have good reliability and validity (Wechsler, 2003). In the FASD literature, it is frequently used to assess working memory ability, and it has also been found to be sensitive to the effects of PAE (Rasmussen, 2005). During this task, and in contrast to the *digit span forwards* condition, the participant is required to repeat a string of digits previously read by the examiner in a backward order, i.e., reversing the order the examiner used to read the stimuli.

Goal setting. The Tower of London (TOL; Culbertson & Zillmer, 2001) test was administered to assess goal setting. This task has been shown to be a reliable and valid measurement of children's planning and problem-solving ability (Culbertson & Zillmer, 2001). The test consists of two boards with pegs on them. The examiner uses three different coloured beads to make a specific pattern on one of the boards, out of sight of the participant. The pattern is then shown to the participant, who is required to align the beads on the second board in the same pattern in as few moves as possible and without breaking particular rules constricting the movement of the beads.

Attentional control. The *Rubia Stop Task* (Rubia, Oosterlaan, Sergeant, Brandeis, & van Leeuwen, 1998) is a computerised test used to assess the domain of attentional control. This task uses a stop-task paradigm, which is considered a gold standard measure of response inhibition when studying children with ADHD. Furthermore, in clinical settings, it has been shown to be a reliable measure of response inhibition in children (Nigg, 2005). Pictures of an aeroplane, pointing either to the left or to the right, are presented on a computer screen. The child is instructed to press the corresponding arrow key on the keyboard when the aeroplane appears on the screen. Occasionally, the aeroplane is presented and then explodes; when this occurs the child is instructed not to press any keys. The explosions occur at random intervals and after varying durations of the aeroplane being visible, thus making the occurrence of the explosion unpredictable, and therefore requiring the inhibition of a learned motor response.

Attentional control was further assessed using the inhibition condition of the *Colour-Word Interference* subtests of the Delis-Kaplan Executive Function System (D-KEFS, Delis, Kaplan, & Kramer, 2001). This subtest is based on the Stroop (1935/1992) test and measures an individual's ability to inhibit an overlearned response. The Colour-Word Interference subtest has been used previously to assess response inhibition in children with FASD (Mattson et al., 1999; Rasmussen & Bisanz, 2009). This test has good reliability and validity (Delis, et al., 2001). During the inhibition condition of the Colour-Word Interference test, the participant is required to inhibit an initial response (i.e., reading the printed name of a colour) and to instead name the ink colour in which the word is printed.

Cognitive flexibility. The Children's Colour Trails Test (CCTT; Llorente, Williams, Satz, & D'Elia, 2003) assesses sustained attention and sequencing, which generalise to the domain of cognitive flexibility (Anderson, 2002). In the first trial of the CCTT, the participant is required to connect a series of circles with numbers inside them in the correct ascending

number sequence, as quickly as possible, while trying not to make any mistakes. During the second trial of the CCTT, the participant is again required to connect a series of circles. However, during this trial each number is displayed twice, either in a pink or a yellow circle. Here, the participant needs to connect the circles in the correct numerical order while always alternating the colour of the circle, i.e., switching between yellow and pink stimuli. The CCTT is designed to minimise the effect of language and has been shown to have both good reliability and validity and is, furthermore, valid cross-culturally (Llorente et al, 2003). It is, thus, highly suitable for the current heterogeneous setting, in its original form with an Afrikaans translation of the instructions.

The inhibition/switching condition of the *Colour-Word Interference* subtests of the D-KEFS (Delis, et al., 2001) was part of the cognitive flexibility assessment. During this condition of the subtest the participant needs to either (1) inhibit an over-learned response of reading a word by naming the colour it is presented in instead (inhibition component) and (2) switch to reading the actual word, rather than naming its ink colour, when the stimulus is presented in a box (switching component).

As detailed above, for assessing attentional control and cognitive flexibility, two EF tests were administered. Z-scores were generated of the raw scores of the two tasks to create a composite score for each domain.

Verbal Fluency. Instead of including an information processing domain, verbal fluency was considered separately considering evidence that children with FASD have deficits in verbal fluency specifically (Jacobson, Jacobson, Sokol, Chiodo et al., 1998; Kodituwakku et al., 1995). Also, fluency assesses abilities that are not specific or exclusive to just one EF domain and could also, for example, be assigned to the cognitive flexibility domain based on its divided attention and working memory component. Thus, instead of assigning fluency to

the domain of information processing as suggested by Anderson (2002), performance on three verbal fluency tasks (see below) were grouped together and analysed separately as a standalone measure of EF.

The *Verbal Fluency* subtest of the D-KEFS (Delis et al., 2001) was used to assess information processing ability. The D-KEFS subtests have been normed for individuals between the ages of 8 and 89 years and have been shown to have a high content validity for the assessment of EF (Delis et al., 2001). The Verbal Fluency subtest measures both lexical and semantic generativity and was specifically selected for this research as it has been shown that verbal ability, and the ability to inhibit pre-potent responses, may impact on ToM (Hill, 2004). In addition, the D-KEFS verbal fluency test has previously been used to assess verbal generativity in children with FASD (Mattson et al., 1999; Rasmussen & Bisanz, 2009).

This tasks consists of three subtests: letter fluency, category fluency and switching. For each subtest the participants are given a 60-second time limit within which to generate as many responses as possible. For letter fluency, participants need to consecutively generate as many words as they can think of starting with the letters 'F', 'A' and 'S'.

Considering the aforementioned cultural bias and multi-lingual research setting, letter fluency required some adaptation. For this subtest, a letterset proven to be more suitable for the Afrikaans language was chosen. The original English letterset consisting of the letters 'F', 'A' and 'S', was replaced with the letters 'B', 'L' and 'S' for the Afrikaans speakers. These letters have been shown to be more valid for assessment of verbal fluency in the Afrikaans language (Ferrett et al., 2014). For the category fluency subtest, participants need to generate words related to a particular semantic category, namely either 'animal' or 'boys/girls names'. For the switching condition, participants are required to generate as many words as possible while switching between the categories, *fruit and vegetables* and *items of clothing*.

Behavioural measures. Two measures were used to gather information on each participant's behaviour and psychiatric state. These were (1) the Schedule for Affective Disorders and Schizophrenia for School Aged Children Lifetime Version (K-SADS-PL; Kaufman, Birmaher, Brent, Rao, & Ryan, 1996) and (2) the Disruptive Behaviour Disorders Inventory **(**DBD; Pelham, Gangy, Greenslade, & Milich, 1992). The K-SADS interviews were administered by the longitudinal study's paediatrician (C Molteno, MD) to the mother to obtain information relevant to a diagnosis of ADHD, Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD). An ADHD diagnosis enabled inclusion of the measure as a potential confounder to determine whether the observed effects in each of the three studies were attributable to PAE or ADHD. It is important to note that only very few, if any of the children in this community who meet criteria for ADHD are treated with Ritalin or other psychostimulant medication for ADHD, so their performances on these tests are not influenced by any of the medications frequently used in the U.S. to treat ADHD. Based on caregiver interviews, only two participants in the current sample (1 HE and 1 control participant) were reported to be on Ritalin.

K-SADS-PL. The K-SADS-PL (Kaufman et al., 1996) was administered to the mother to determine past and current psychopathological episodes in children and adolescents. This semi-structured interview is based on the DSM-III and DSM-IV diagnostic criteria for a wide range of psychiatric disorders including separation anxiety disorder, schizophrenia, OD, CD, ADHD, post-traumatic stress disorder, alcohol and substance abuse. The K-SADS-PL is widely used in clinical and research settings and has been shown to have good reliability and validity as a diagnostic tool (see e.g., Kaufman et al., 1997; Lauth, Arnkelsson, Magnusson, Skarpeoinsson, Ferraru, & Petursson, 2010). The K-SADS-PL has also previously been administered in other South African-based research studies (see e.g., Ferrett, Carey, Thomas,

Tapert & Fein, 2010; Ferrett et al., 2014).

DBD. The DBD (Pelham et al., 1992) is a 45-item screening questionnaire that can be completed by a child/adolescent's teacher or parent. It is designed to capture information about symptoms of ADHD, ODD and CD. On the DBD, an individual's behaviour is rated using the scale's four measures pertaining to inattention and hyperactivity, ODD and CD. For each question the informant rates a behaviour either occurring as "not at all", "just a little", "pretty much" or "very much". Questions to which the informant does not know the answer are responded to with "DK" (do not know). The DBD rating scale has been shown to have good reliability and validity across a variety of participant groups (Friedman-Weieneth, Doctoroff, Harvey, & Goldstein, 2009; Silva et al., 2005). The DBD has previously been used with the larger longitudinal cohort to examine the protective effects of dehydrogenase-*ADH1B*3* allele on attention and behaviour problems in adolescents exposed to alcohol during pregnancy (Dodge et al., 2014). It was found that teacher reported symptoms of inattention were related to PAE, after controlling for potential confounders. However, this effect was only seen in children born to mothers without at least one copy of the protective *ADH1B*3* allele. For the current project, composite scores for each of the DBD factors were constructed by transforming the raw scores to standard scores and taking their average.

Continuous alcohol measures. The information collected during the prenatal visits to the CDRL was used to document the PAE history for each participant. The following continuous alcohol measures were generated to study the effect of alcohol exposure independent of FASD diagnosis: (1) AA/day across pregnancy (i.e., how much AA was consumed per day across the duration of the pregnancy); (2) AA/drinking day across pregnancy (i.e., how much AA was consumed on days the mother actually consumed alcohol); (3) frequency of drinking days across pregnancy (i.e., number of drinking days

across the pregnancy). Similarly, other drug use (i.e., marijuana ("Dagga"), cocaine, heroin, and methaqualone ("Mandrax") and smoking during pregnancy was also documented and considered as potential confounders of the effects of alcohol exposure.

Procedure

For Studies I and II, children and their primary caregiver were transported, in a van owned by the study, from their home to the CDRL at the UCT. The neuropsychological assessments were conducted over 2 days at the laboratory. On each day, participants and their caregivers received breakfast, a snack and lunch at the CDRL. Each child was tested individually while the mother was interviewed in a nearby room regarding the child's school and health history, demographics, the mother's current alcohol, smoking, and drug use by the senior longitudinal cohort pediatrician (C Molteno). Testing was conducted in Afrikaans or English, depending on the primary language of instruction in the child's school. Examiners were blind regarding fetal alcohol exposure history and FASD and psychiatric diagnoses.

Cognitive measures. Measures of IQ and EF were collected during the 2-day visit of Study I and utilised for analyses across all three studies. Standardised scoring procedures were followed, as outlined in the testing manual of each measure. While a standardised IQ score was generated for the general intelligence assessment using the WISC-IV manual, participant's scores from the diagnostic groups were compared to the scores of control children, rather than against the Western norms provided in the WISC-IV manual. Similarly, for the EF measures, each participant's raw score was used for group comparisons. The measures unique to each study of the current research project were administered as part of a larger neuropsychological test battery during the 2-day visit at the time each study was conducted. Due to the location of the neuroimaging testing centre, the procedure for Study III differed slightly and is outlined in the *Chapter Six* (pp. 149-150).

Behavioural measures. Each child was rated on the DBD by their teacher. Symptom counts were computed separately by the longitudinal cohort's paediatrician for inattention and hyperactivity by totalling how many of the nine DSM-IV behavioural criteria for each ADHD subtype were endorsed as ''often'' or ''very often'' by the teacher. In addition, the paediatrician administered the ADHD, ODD and CD modules of the K-SADS to the mothers for assessing ADHD and externalising behaviours.

The final ADHD diagnoses were made as part of a case conference conducted by C Molteno and S Jacobson. They used research criteria developed in collaboration with R Klorman and J Nigg, two clinical psychologists widely recognized for their expertise in ADHD research. An ADHD classification was assigned if (a) at least 6 of the 9 symptoms for inattention and/or 6 of the 9 symptoms for hyperactivity/impulsivity was endorsed (''pretty much'' or ''very much'' true of child), and (b) some impairment (≥2 ADHD symptoms) was reported by age 7 years and in two or more settings (operationally defined in terms of reports from at least two informants—mother/primary caregiver, examiner). An ODD diagnosis required at least four unique symptoms reported by parents and/or teachers; CD required three.

Investigating the potential presence of these disorders provided information about each child's behavioural profile. The behavioural problems associated with prenatal alcohol exposure often resemble those seen in other disorders. For example, ADHD is often co-morbid with FASD. These diagnoses were made to investigate whether the association between social-cognitive abilities and PAE in these participants were observed independently of an ADHD diagnosis.

Ethical considerations. The current research followed the University of Cape Town's code specified for Research Involving Human Subjects and guidelines provided in the

Declaration of Helsinki of 2000. Permission to conduct the research with the longitudinal cohort was obtained from the University of Cape Town's Faculty of Health Sciences Human Research Ethics Committee (see Appendix A) and the Human Investigation Committee at Wayne State University (see Appendix B). Informed consent was obtained from the mothers at recruitment and at the child assessment visits; assent was obtained from the children. Children received a small age-appropriate gift, and the primary caregiver received compensation consistent with guidelines from the UCT ethics committee.

Informed consent and assent. All participants (i.e., mothers, primary caregivers and children) were informed that they may choose to discontinue taking part in the longitudinal study or leave testing at any time without any repercussions or penalties. If they had any questions or concerns, these were addressed by the investigator or research staff. In addition, participants/primary caregivers were provided with the contact details of the Principal Investigators and the Ethics Committees, should they have wished to direct queries to them. For each new phase of testing, informed consent and assent was obtained from the mother/primary caregiver and child. The content of the corresponding forms was approved by the Ethics Committees of the University of Cape Town and Wayne State University. All forms were translated into Afrikaans by a native Afrikaans speaker and were administered in either English or Afrikaans, depending on the mother/primary caregiver/child's preference or language ability. A copy of the English (Appendix C) and Afrikaans (Appendix D) informed consent forms used for all three studies and the English (Appendix E) and Afrikaans (Appendix F) assent forms for Study III are attached as Appendices.

Confidentiality. To ensure anonymity and to restrict access to any sensitive information, all test results from the behavioural and fMRI assessments and all information collected during the interview process, have been kept strictly confidential. All data collected for a

participant were assigned a code unique to that participant. Each participant's files are stored in locked cabinets at the CDRL at the University of Cape Town and at Wayne State University. Only authorised personnel have access to this documentation, and information is only given out upon the written permission of the mother/primary caregiver. No identifiable information or participant names will be used in publications. Any photographic/video material that may have been collected during the testing sessions may be used for scientific or teaching purposes, but only with prior written consent of the mother/primary caregiver. To protect participants in our longitudinal cohort, we are required by law to report any evidence of child abuse or neglect to the relevant authorities. We adhere strictly to these guidelines.

Benefits. Participation in the longitudinal project, and in the research described here does not directly benefit the participants involved. Overall, the longitudinal study aims to provide knowledge and new insight to better understand the effects of PAE on cognitive development. The current research studies were aimed at understanding the underlying mechanisms of social-cognitive impairments related to PAE in children and will hopefully benefit individuals with FASD in future. Referrals are made for medical problems, such as lead exposure, iron deficiency, TB, malnutrition, or for educational remediation or psychological problems or help with alcohol or drug abuse, if requested by the parent. Furthermore, in the case of a positive diagnosis of any of three psychiatric disorders (ADHD, CD or ODD) measured by the KSADS-PL and DBD, it was discussed with the mother/primary caregiver. If necessary, the child was referred to the appropriate health centre for further investigation and/or treatment. The diagnosis was not communicated to the child's teacher or school unless the mother/primary caregiver requested this in writing.

Costs and compensation. Participation in this research bears no costs to the mother/primary caregiver or child. A monetary compensation of ZAR150 was given to the

mother/primary caregiver at each research visit, and the child was given a small gift. Participants were transported to and from their residence and received breakfast, a small snack and lunch at the research laboratory to avoid incurring any food and transport costs. With the exception of Study III (which was shorter), testing took place over 2 days to avoid possible fatigue effects. During each testing day, the participant was encouraged to take breaks as often as needed, with a mandatory lunch break in between. The average time taken to administer the full test batteries, which included the measures of Study I and Study II, was about 3 hours over 2 days including breaks and lunch. The assessment during Study III, including both participant preparation and the actual scanner session, lasted about 2.5 hours.

To review, the current research project utilises concepts embedded in social information processing in an attempt to understand the social-cognitive impairments present in individuals with PAE. Each of the three studies involved participants from an established prospectively recruited longitudinal cohort in Cape Town, consisting of children with a diagnosis of FASD and typically developing children from the same community. There was significant overlap in the measures and procedures employed across the three studies, in particular regarding the cognitive measures administered, and these were thus collectively summarised above. Now, that the context of the current research setting has been established, the proceeding chapters will present the findings of each of the studies making up this dissertation.

CHAPTER FOUR: Study I – An empirical investigation of Theory of mind in children with FASD

Introduction

Research examining detrimental effects of alcohol on the developing brain has documented a broad range of cognitive and behavioural deficits in children with fetal alcohol spectrum disorders (FASD; Carmichael Olson et al., 1997), including IQ and attention deficits; poorer learning and memory, and impaired EF (Mattson et al., 2011). As outlined in the previous chapters, social and behavioural problems have also been reported in children with FASD. In this chapter, I first briefly summarise the limited empirical evidence on social-cognitive deficits in individuals with FASD, which was reviewed in detail in *Chapter Two* and provided the rational for the current research. I then present the methods of the first study of this dissertation, which provided the initial examination of social-cognitive ability in this FASD cohort. Finally, I present the analyses and results, which considered between-group differences based on FASD diagnosis as well as the effect of PAE independent of FASD diagnosis, based on continuous measures of prenatal alcohol consumption. The chapter closes with a discussion of this study's findings.

Social cognition is based on observation and interpretation of social stimuli, including affective expressions. Emotions of the self and others play an essential role in interpreting social situations that ultimately shape social knowledge and interactions (Halberstadt et al., 2001). Although deficits in social information processing and social problem-solving skills have frequently been described by clinicians, teachers, and caregivers (Roebuck et al., 1999), social-cognitive impairment in FASD has been documented in only a few recent studies, and

specific aspects of social cognition impaired in children with FASD are not well-understood. To summarise, one study found that scores of adaptive behaviour, such as daily living and communication skills in children with FAS exceeded what could be explained by low IQ scores (Thomas et al., 1998). In another study, parents reported more socially problematic, delinquent, and aggressive behaviours in alcohol-exposed children than controls (Mattson & Riley, 2000). Similarly, teachers reported more problems in these domains in children with PAE at 7.5 and 14 years (Dodge et al., 2014; Jacobson, Carr et al., 2006). In a study using video vignettes of children in problematic social situations, alcohol-exposed children exhibited more dysfunctional social information processing than controls (McGee et al., 2009). Another study found that adolescents with heavy PAE showed impairment in everyday social problem solving, deficits that were related to poorer EF (McGee et al., 2008). A deficit in ToM, a domain of social-cognitive ability related to social behaviour, may play a role in the development of these children's poor social skills (Thomas et al., 1998).

Overview of ToM Research

ToM is defined as the cognitive capacity to recognize and understand mental states of others and to predict their behaviour (Baron-Cohen et al., 1994). Because social interaction requires complex and flexible behavioural initiations and responses, ToM is considered a crucial component of effective social communication (Baron-Cohen, Wheelwright, Scahill et al., 2001). The processes underlying ToM can be characterized by (1) epistemic inferences, which a person makes about another person's beliefs , and (2) affective inferences, which enable a person to comprehend and interpret the emotional states of others (Stone, Baron-Cohen, & Knight 1998). ToM is said to exist as a distinct cognitive domain not dependent on overall general intelligence (Baron-Cohen et al., 1994), which can be selectively impaired even when other aspects of cognition, such as language, remain intact (Stone et al., 1998).

Typical developmental trajectory of ToM. ToM is considered to be a mental process, which develops and matures post-natally and its onset is typically between the ages of 3-5 years (Stone et al., 1998; Wellmann, Cross, & Watson, 2001). In the past, the shift to understanding mental states, (i.e., the onset of ToM development) has frequently been equated to passing false-belief tasks (Callaghan et al., 2005). This task has become the prototypical tool in assessing ToM ability and has contributed to the notion that ToM follows a certain developmental trajectory.

Early ToM-related developmental milestones emerge around 18 months through the expression of (1) joint attention (i.e., directing one's attention by e.g., looking at an object another person is looking at) and (2) proto-declarative pointing (i.e., pointing at an object to draw another's attention to an object; Baron-Cohen, 1995). The next marker of early ToM development becomes apparent around 18-24 months, when toddlers begin to engage in pretend play (i.e., dissociating between pretend and reality; Leslie, 1987). This milestone is followed by the development of the ability to understand the concept of desire (e.g., "Mary wants ice cream"; Wellman & Wolley, 1990) at around 2 years. The ability to understand other peoples' beliefs emerges next. Children aged 3-5 years, begin to understand their own false-beliefs and those of others, and also start distinguishing between appearance and reality (Bibby & McDonald, 2005; Wellman, 1990). The ability to understand the concept of second-order false belief (i.e., understanding that another person can hold a belief different to one's own) develops between ages 5 and 7 (Perner & Wimmer, 1985; Wellman & Liu, 2004). Dissociating between jokes and lies, comprehending metaphors and non-literal language such as sarcasm and irony generally begins to develop around 7 years, when children start to understand that a speaker's statement may not be meant literally but they still mostly fail to understand the actual intent. At age 9, only about 25% of typically developing children fully

understand the alternative non-literal intent of sarcasm and irony (Filippova & Astington, 2008). Therefore, development of understanding non-literal language, such as irony and sarcasm, is considered an advanced aspect of social-cognitive development. Much like the more basic ToM abilities, such as false-belief, comprehension of non-literal language unfolds progressively throughout development (Peterson, Wellman & Slaughter, 2012; Wellman & Liu, 2004).

The recognition of social faux-pas (i.e., a person saying something they should not have said not realising they should not have; Stone et al., 1998) develops around 9-11 years. The comprehension of a faux-pas is a complex process requiring the ability to represent two mental states, (1) the mental states of the person committing the faux-pas and (2) inferring the feelings of the person at who the faux-pas was directed (Baron-Cohen, O'Riordan, Stone, Jones, & Plaisted, 1999).

The concept to infer complex mental states of others develops after the understanding of faux pas and is considered an advanced ToM ability. Research has shown that eyes appear to be particularly relevant to the decoding of facial social cues because the display of different eye expressions helps us decode the specific emotion portrayed. Hence, this type of facial cue processing enables mental state reasoning, which is crucial in social communication (Sabbagh, 2004; Tager-Flusberg, 2001). Moreover, typically developing participants have been shown to decode complex mental states just as accurately when processing the whole face or when shown only the eye regions (Baron-Cohen, Wheelwright, & Jolliffe, 1997).

Typical measures employed in ToM research. A critical and prototypical task used to assess ToM ability is the false-belief test, which is frequently used to examine ToM in young children. This task is designed to measure a person's ability to infer the epistemic mental states of others (i.e., understanding that a person can hold a belief that does not necessarily

correspond to the actual situation; Wimmer & Perner, 1983). False-belief is comprised of two complexity levels, referred to as a first- and second-order false belief. The more basic level, first-order false belief, is described as the ability to understand that another person can hold a mental state that is different to reality. Second-order false belief, is more complex, and refers to the ability to conceptualise that another person can have a belief about what a second person is thinking. Typically developing children have been shown to develop an understanding of false belief during pre-school years (Wellman, 2012). Although this development can vary up to 2 years across different communities (Liu, Wellman, Tardif, & Sabbagh, 2008), the comprehension of false belief can possibly be considered a universal development milestone (Wellman, 2012). However, passing false-belief tests is only one of the many milestones in ToM development. Therefore, inferring that ToM ability has fully developed based solely on the performance of one task, is erroneous

Today it is well-established that ToM ability continues to develop as the child matures and moves beyond the scope of understanding false-beliefs (Wellman et al., 2001). ToM includes the development of higher order abilities necessary for navigating more complex social scenarios by understanding mental concepts such as desires and intentions. Thus, these more complex aspects of ToM need to also be examined, especially when targeting older children. The faux-pas test, is a task typically aimed to assess older children aged 7-11 years. This task examines the ability to identify and understand socially awkward situations. In contrast, to the false-belief task, which only involves epistemic inferences, the ability to recognise and comprehend a faux-pas requires both affective and epistemic inferences. This test was first developed to assess ToM ability in both typically developing and autistic children (Baron-Cohen, O'Riordan, et al., 1999). This task is frequently used to assess more advanced ToM ability or social cognition and has been applied across various clinical groups

including individuals with psychosis (see e.g., Zhang et al., 2015), schizophrenia (see e.g., Altamura et al., 2015), borderline personality disorder (BPD; see e.g., Petersen, Brakoulias, & Langdon, 2016), frontotemporal dementia (see e.g., Bertoux, O'Callaghan, Dubois, & Hornberger, 2015), in children with ADHD (see e.g., Mary et al., 2016) and in adults (see e.g., Spek, Scholte, & Van Berckelaer-Onnes, 2010) and children with autism (see e.g., Baron-Cohen, O'Riordan et al., 1999).

Another advanced ToM test is the *Strange Stories* task (Happé, 1994), which is a compilation of stories describing everyday scenarios. Each story is constructed to assess the understanding of *why* a protagonist gave a response that is not literally true. The themes of the stories probe concepts such as irony, lies, jokes, pretend, double-bluff and figure of speech. For each story, the context of the situation is provided as this dictates the protagonist's response and affects the interpretation thereof. Therefore, the *Strange Stories* task assesses a person's ability to correctly interpret a social situation by being required to explain the motivation behind a protagonist's response. Since its development, the *Strange Stories* task has been used to examine more advanced ToM abilities in various clinical conditions, for example, in individuals with alcohol use disorder (Bosco, Capozzi, Colle, Marostica, & Tirassa, 2014), to examine implicit and explicit ToM ability in autism (Schuwerk, Vuori, & Sodian, 2015) and to examine deficits in social cognition in children with epilepsy (Lew et al., 2015).

Another advanced ToM task developed to measure mental attribution state in autism is the Reading the Mind in the Eyes task (RME; Baron-Cohen, Wheelwright, Hill et al., 2001). The RME involves the processing of complex facial emotion recognition based solely on eye expressions and is able to identify subtle impairments in social cognition (Baron-Cohen, Wheelwright, Hill et al., 2001). Performance on the RME has been shown to be independent

of both IQ (Baron-Cohen, Wheelwright, Hill et al., 2001) and EF (Ahmed & Miller, 2011). However, although the RME is considered an advanced ToM test, it only involves the attribution part of ToM and does not include inferring the content of the attributed mental state (Baron-Cohen, Wheelwright, Hill et al., 2001). The RME has been used and evaluated in over 250 studies (Vellante et al., 2013), extending its application beyond the field of autism and has been used, for example, to examine the mentalizing ability in BPD (Petersen et al., 2016), the EF and attentional contributions to ToM in ADHD (Mary et al., 2016) and deficits in social cognition in children with epilepsy (Lew et al., 2015).

ToM consists of a set of interrelated skills that emerge sequentially, following a developmental progression as the child matures. However, some research also suggests variation in ToM development across different cultures (Wellman, 2012). Cultural variation may be attributable to differences in traditional cultural upbringing, specifically the different focuses of collectivist versus individualist cultural contexts (Slaughter & Perez-Zapata, 2014). Until fairly recently, most ToM research used tasks that only measure single aspects of ToM frequently using the prototypical false belief task. However, given what is known about developmental trajectory of ToM, when examining this ability in children, a developmentally sequenced ToM test battery needs to be used (Steele, Joseph & Tager-Flusberg, 2003).

ToM and FASD

To date, only three previous studies have examined ToM in FASD. Rasmussen et al. (2009) found that children (4-8 years) with FASD performed more poorly on false belief tasks than controls. Because poorer response inhibition by children with FASD predicted their ToM performance, the authors concluded that the ToM deficit was attributable to difficulties with inhibitory control. Greenbaum et al. (2009) found that children (6-13 years) with FASD were poorer at identifying facial emotions and predicting a protagonist's behaviour and

corresponding facial expression compared to children with ADHD and healthy controls. These social skills were related to performance on tasks of emotion processing but not to performance on a ToM second-order false-belief task. A recent study comparing children with FASD to controls (aged 6-16) detected no between-group differences on the NEPSY-II Social Perception subtest (Rasmussen et al., 2013). Although these studies focused on specific aspects of ToM, none employed a comprehensive developmentally sensitive ToM battery.

Considering the effect of other PAE-related deficits. Widespread EF deficits, including difficulties with inhibition, cognitive flexibility and working memory, are characteristic of the cognitive profile of children with FASD (Burden et al., 2005; Rasmussen et al., 2011). Both neuropsychological (Mattson et al., 1998; Rasmussen et al., 2011) and neuroimaging (O'Hare et al., 2009) studies have demonstrated that the range of EF impairments present in FASD is associated with structural and functional cortical damage related directly to PAE. Impairments in EF also exist in children with PAE who lack the characteristic physical features (Connor et al., 2000; Mattson et al., 1998). Moreover, EF contributes to social-cognitive functioning and ToM ability (Fahie & Symons, 2003). Thus, executive dysfunction may diminish social interaction and negatively affect a child's developing understanding of the mind. Therefore, EF and ToM may indeed be related but ToM may, furthermore, be governed by the quality and quantity of a child's social interaction.

Engaging and solving any given cognitive tasks, requires both conceptual understanding and other non-focal cognitive skills. For example, to demonstrate understanding of a false-belief task an individual needs to (1) mentally represent another person's belief (i.e., conceptual understanding), and at the same time, (2) remember the details of the given situation (with the help of WM), and (3) focus his/her attention on the problem at hand (using attention and inhibitory control to suppress any other irrelevant

perspectives). Therefore, because PAE adversely impacts EF over and above low IQ (Rasmussen, 2005) and predicts social skills deficits (Schonfeld et al., 2006), it is important to determine the degree to which alcohol-related ToM deficits are due to EF impairment.

Similarly, ADHD has also been shown to have a high co-morbidity with FASD, and as many as 70% of children with PAE also receive a diagnosis of ADHD (Berg, Kinsey, Lutke, & Wheway, 1995; Coles et al., 1997; O'Malley & Nanson, 2002). Furthermore, children with ADHD have been described as demonstrating similar social deficits as children with FASD (Greenbaum et al., 2009). These social impairments include troubled relationships with family and peers (Mash & Johnston, 1983; Pelham & Bender, 1982) and difficulties grasping the consequences of their actions (Barkley, 1998; Dodge, 1986). Therefore, because ADHD is commonly diagnosed in children with FASD (Jacobson, Dodge et al., 2011) and has an impact on ToM (Uekermann et al., 2010), the extent to which these deficits are attributable to ADHD were also examined.

ToM Research in South Africa

If the sequence in which ToM develops is indeed to some degree universal (Liu, Wellman, Tardif, & Sabbagh, 2008; Wellman et al., 2001), developmental patterns in children from different countries or communities should follow a similar trajectory. Following this notion, various studies have examined ToM ability in non-Western populations (see e.g. Liu et al., 2008; Naito, 2003; Wellman et al., 2001), yet only two ToM South African studies exist. Van Staden (2010) investigated ToM ability in native signing, late-signing and orally trained deaf children in the Free State Province of South Africa. A more recent study, using a developmentally sensitive ToM battery found that Cape Town children with high-functioning autism and Asperger's syndrome (AS) showed delayed ToM development as performance improved with age (Hoogenhout & Malcolm-Smith, 2014). They also showed that within the

autism spectrum disorders group, the highest proportion of individuals passing the Faux-Pas and Strange Stories task were those with AS. Importantly, the performance of the typically developing control participants evidenced a similar developmental trajectory to that described in previous literature (e.g. Wellman et al., 2001). However, ToM ability in South African children with FASD has not yet been assessed.

Rationale for the Current Study

Impairment in social behaviour among children with FASD is evident. Yet, the research reviewed above demonstrates that little is known about the effects of FASD on ToM ability. Also, the notion that social impairment becomes more pronounced as children with FASD age, needs to be further explored (Carmichael Olsen et al., 1997; Streissguth et al., 1991). To my knowledge, no study has to date adequately investigated ToM ability in older, pre-teen adolescent children with FASD. Furthermore, considering both developmental trajectory and the review of the measurement of ToM above, it becomes clear that the selection of a range of appropriate ToM measures is crucial.

This study is the first to investigate ToM in children along the fetal alcohol spectrum using a developmentally-sensitive ToM test battery. I administered this battery, as well as IQ and EF tests, to a sample of school-age children with and without heavy PAE. The aim of the study was to examine whether impairments in ToM, including affect recognition, can explain the mechanisms underlying the social-cognitive impairments seen in children with FASD. The primary goals were to examine the degree to which: (1) FASD diagnosis and a continuous measure of PAE obtained during pregnancy are related to poorer ToM; (2) prenatal alcohol-related effects on ToM are attributable to lower IQ or are mediated by alcohol-related deficits in EF; and (3) observed ToM effects are attributable to ADHD. Hence, the following questions were investigated: Do young adolescents with FASD demonstrate

impairment in ToM and affect recognition tasks when compared to matched healthy controls? And if so, are there significant group differences between the FASD sub-types? Furthermore, how does the effect of prenatal alcohol exposure contribute to social-cognitive impairments independent of FASD diagnosis?

Specifically, the predictions for Study I were:

1. Young adolescents with FASD will demonstrate deficits on ToM tasks in comparison to matched healthy controls. Moreover, children with FAS will perform worse on ToM tasks compared to children with PFAS who will perform worse than HE children who will perform worse than typically developing individuals (i.e., FAS < PFAS < HE < Controls).
2. Young adolescents with FASD will demonstrate deficits on affect recognition tasks in comparison to matched healthy controls. Furthermore, children with FAS will perform worse on affect recognition tasks compared to children with PFAS, who will perform worse than HE children, who will perform worse than typically developing individuals (i.e., FAS < PFAS < HE < Controls).
3. The potential deficits in ToM and affect recognition in young adolescents with FASD will be present even when general cognitive and executive impairments are controlled.
4. The effect of PAE on ToM and affect recognition will be more sensitive when examined using a measure of continuous alcohol independent of FASD diagnosis.

Methods

Participants

The sample consisted of 63 Cape Coloured children (aged 9-11) born to women recruited into the Cape Town Longitudinal Cohort. For further details on the recruitment of participants and the process of FASD diagnosis, refer to the *Participants* section in *Chapter Three* (pp. 40-42).

Measures

Theory of Mind. To examine the potential deficits in ToM ability in children with FASD, a compilation of established ToM tests was selected for the current study. Although ToM may be a universal ability, tasks created to measure ToM ability may be culturally bound. To ensure that the administered ToM tasks were suitable for a non-Western population, potentially culturally-biased content was adapted for administration in the South Africa. The battery was compiled and adapted for the South African context by the UCT Autism research group (Hoogenhout & Malcolm-Smith, 2014) based on previous research on ToM with children with autism (Happé, 1994; Joseph & Tager–Flusberg, 2004). Modifications for the South African context were minor adjustments in terminology to make the tasks more appropriate for administration to the local population. For example, in a story 'baseball' was changed to 'cricket', 'football' to 'soccer', foreign currency to South African currency, 'lavatory' to 'toilets' etc. These expressions are all terms that South African children are more familiar with. Once adapted all the ToM tasks were then translated into Afrikaans while retaining their culturally-adapted meaning.

To evaluate the performance on specific ToM tasks, the following four measures from the UCT ToM battery were administered: First - and Second-order False Belief, Strange Stories, and Faux-Pas (Faux-Pas Detection and Faux-Pas Comprehension). Examples of the

task stimuli are presented in Table 4.1 and in Appendices C1-C4. To minimize demands on memory and the effect of language ability, the ToM stories were presented with pictures and placed in front of the participant during the tasks administration.

The *First-Order*, also known as the *Location-Change False Belief* task (Baron-Cohen et al., 1985; Steele et al., 2003; Wimmer & Perner, 1983) is comprised of 2 picture stories, which describe how an object is moved from one location to another, while the protagonist is not present in the room. The participant is then asked (1) a control question to probe whether the content of the story was understood, (2) whether the protagonist who was not in the room will know where the object had been moved to, (3) where, upon returning to the room, the protagonist will look for the object, and (4) why the protagonist will look for the object in that location. For an example of the picture stories used, see Appendix G.

The Second-Order False Belief task (Sullivan, Zaitchik, & Tager-Flusberg, 1994) assesses the participant's ability to deduce what a character in a story thinks or knows about what a second character thinks or know. The participant is shown 2 picture stories and then asked a set of three questions: an ignorance question ("Does the character think/know what the second character thinks/knows?"), a belief question ("What does the character think/know about what the second character thinks/knows?") and a justification question ("Why does the character do that?"). As a control measure, to establish whether the participant was indeed attending to the stories, 2 content-related questions also need to be answered. The responses to these questions were scored as correct or incorrect and generated three measures of second-order false belief, namely (1) a *control* measure (compiled of the total score received for the 2 content questions), (2) a *recognition* measure (based on the score for the question probing the identification of the belief), and (3) a *comprehension* measure (based on the score for justifying protagonist's belief). For an example of the picture stories used, see Appendix H.

The *Strange Stories* task (Happé, 1994) assesses the participant's ability to distinguish between literal and non-literal language. In this task, 18 stories (12 ToM and 6 control stories) were placed in front of and read to the participant. Embedded in each of 12 ToM stories is one of the following themes: contrary emotions, irony, lie, pretend, appearance-reality, persuasion, double-bluff, white lie, misunderstanding, joke, figure of speech and forgetting. Following each *ToM strange story*, the participant needs to infer a story character's mental state by explaining the character's behavior. By contrast, following each *control strange story*, the participant needs to make a physical inference. The administration of the ToM stories together with the control stories allows for the assumption that if a participant failed the *ToM strange stories,* yet passed the *control strange stories*, the participant's impairments are related to inferring mental states specifically, and not to general inference making ability (Happé, 1994). For an example of the picture stories used, see Appendix I.

The adapted version of the *Faux Pas* task (Stone et al., 1998) assesses a participant's ability to recognize a social faux pas. In this task, stories of 5 'normal' social interactions and 5 socially-awkward or embarrassing situations were placed in front of and read to the participant. At the end of each story, the participant was asked a series of question, namely 2 *faux-pas control questions* (requesting specific details regarding the content of the story) and a question probing the presence of a faux pas ("Did someone say something they shouldn't have said?"; generating the *faux-pas detection* measure). If the participant identified a faux-pas as being present, further questions were asked. These questions probed the participant's comprehension of (1) the faux pas ("Who said something they shouldn't have said?"), (2) the mental state of the character against who the faux pas was committed ("Why shouldn't they have said it?"), and (3) the mental state of the character speaking ("Why did they say it?").

The score of latter three questions collectively generated a *faux-pas comprehension* measure. For an example of the picture stories used, see Appendix J.

In addition to the tasks administered from the UCT ToM battery, the Contextual Task from the NEPSY-II Social Perception subtest was also administered (Korkman, et al., 2007). The Contextual Task is part of the ToM component from the NEPSY-II and requires affective mental state inference based on a specific context. For a more detailed task description, see Table 4.1.

Facial affective processing. To examine facial emotion processing, which has been shown to be linked to ToM (Buitelaar & van der Wees, 1997; Phillips, Wellman, & Spelke, 2002), the Affect Recognition (AR) subtest from the NEPSY-II Social Perception subtest was also administered (Korkman, et al., 2007). In neuropsychological testing, the NEPSY-II AR subtest is a reliable and valid measure widely used to assess affect recognition (D'Amato & Hartlage, 2008). The four tasks making up the AR subtest, aim to assess the ability to recognize facial emotions from photographic stimuli and does not entail a verbal or language component. Either 4 or 6 photographs of children's faces displaying a different affect were shown to the participant. The participant was instructed to select a picture in which a child appears to feel the same as a child in one of the other photographs displayed. Great care was taken to instruct the participant to choose a picture showing a child who appears to *feel* the same and not one that *looks* the same. Although affect recognition is frequently included as part of ToM, it is investigated here specifically as it is a more basic ability and foundational to ToM and social cognition. Because the NEPSY-II AR subtest, has a memory component in one of its tasks and because this may confound performance on this subtest, EF ability, including working memory, was controlled for.

To further investigate advanced ToM ability, the children's version of the RME test ((Baron-Cohen, Wheelwright, Scahill et al., 2001)C was used to assess the child's ability to recognize emotional expressions depicted in photographs of the eye region, including simple and basic emotional expressions (e.g., sad), personality attributes (e.g., kind), and complex mental states (e.g., coercion; see Figure 4.1). RME has been validated as an advanced test of ToM, which can identify subtle impairments in social cognition. It is also the only ToM test with no ceiling effects in healthy adults (Baron-Cohen, Wheelwright, Hill et al., 2001). The RME is reliable and valid and normed for children 6 years and older (Baron-Cohen, Jolliffe, Mortimore & Robertson, 1997). In the children's version of the RME task, a participant was shown 28 black and white photographs, showing just the eye regions of males and females. The participant then needed to choose 1 of 4 words (1 target and 3 foil words), presented together with the photographic stimuli, which best depicted what the person who's eye regions were shown may be feeling or thinking. Both cognitive and affective (basic emotions) words are presented.

Only basic emotions have been shown to be universal in terms of facial expressions. Hence, there is a difference between reading emotions directly from the face, as is the case in affect recognition task, and inferring mental states, as is the case in the RME task. Many epistemic inference tasks rely on a range of intact cognitive abilities, for example, memory and problem-solving abilities (Stone et al., 1998). However, the RME task provides a purer measurement of mental inferential ability because it does not rely on naming ability, memory capacity, or problem-solving skills. In other words, RME investigates a fundamental aspect of social cognition that is not assessed in any of the other ToM tasks outlined above.

Table 4.1

Theory of Mind Battery

Theory of Mind measures	Description
False Belief	Assesses the ability to infer epistemic mental states. Develops by 4-6 years of age. Administered as a set of picture stories.
1st-order False Belief[a]	Assesses the ability to infer that others can have mental states that differ from reality. Two picture stories are presented to the participant during which an object is moved while the main character is not present. The participant is asked whether the main character, who was previously absent, will know where the object is, where the character will look for the object and whether the character will look there. Example of a 1st-order False Belief story: *One day, Emma is in the kitchen eating a chocolate bar. Then Emma's mom comes in and says, "Emma, put away that chocolate. It is time to do your chores." "Okay," says Emma, "but when I come back I'm going to finish eating my chocolate." Emma puts her chocolate in the drawer and leaves to go do her chores. It is a very hot day. Mom is afraid that the chocolate will melt. Mom takes the chocolate form the drawer and puts it in the fridge. Later on, Emma comes back into the kitchen and wants to eat her chocolate.*
2nd-order False Belief[a]	Assesses the ability to conceptualize what another person thinks a second person thinks. Two picture stories are presented to the participant and are followed by three questions: (1) an ignorance question, e.g., does the character think/know what the second character thinks/knows; (2) a belief question, e.g., what does the character think/know about what the second character thinks/knows; and (3) a justification question. Example of a 2nd-order false belief story: *John and Mary are in the park when they see an ice-cream truck. Mary would like to buy an ice-cream but has no money with her. The ice-cream man tells her to go home and get her money because he will be staying in the park all day. Mary goes home, and John stays in the park. Then the ice-cream man John tells he is moving to the church. He drives off, and John goes home. On his way to the church, the ice-cream man meets Mary and tells her where he is going. They arrange to meet at the church, so Mary can buy her ice-cream. Later John goes to Mary's house. Her sister says she has gone to buy ice-cream.*
Strange Stories[a]	Assesses the ability to infer the motivation behind responses that are not literally true. Administered as a set of 18 stories (12

	test and 6 control stories); each test story assesses the understanding of a different concept, namely: lie, white lie, joke, pretend, double-bluff, persuasion, forgetting, misunderstanding, figure of speech, appearance-reality, irony and contrary emotions. This test assesses the participant's ability to consider *why* the protagonist behaved in a certain way. Example of a strange story test story: *Katie and Emma are playing in the house. Emma picks up a banana from the fruit bowl and holds it up to her ear. She says to Katie, "Look! This banana is a telephone!"*
Faux-Pas[a]	Assesses the ability to recognize a socially awkward situation. Develops by 7-11 years of age. Administered as a set of 10 stories (5 control and 5 test stories), with either a normal social event (control story) or an awkward or embarrassing event (test story). This task yields 2 variables: Faux-Pas detection (i.e., the ability to recognise when a faux-pas is present, e.g., did someone say something they should not have said?) and Faux-Pas comprehension (i.e., the ability to understand/explain a faux-pas, e.g., why should they not have said it?). Example of a faux-pas test story: *Kim helped her mom make an apple pie for her uncle when he came to visit. She carried it out of the kitchen. "I made it just for you", said Kim. "Mmm", replied Uncle Tom, "That looks lovely. I love pies, except for apple, of course!"*
NEPSY-II[b]	Standardized measure providing a reliable assessment of neuropsychological functioning in children aged 3-16 years.
Contextual task	Assesses the ability to comprehend the relation between a felt emotion and the display of the corresponding facial affect, depicted across various situations. Administration of a set of pictorial social scenes where one of four photographs needs to be chosen that best shows what a person in the scene is feeling
Affect Recognition	Assesses the ability to recognize emotions from facial expressions. Administration of a set of photographs of children displaying a certain emotion need to be matched to photographs with children with the same affect.
Reading the Mind in the Eyes Child Version[c]	Assesses the ability to infer complex mental states of others from their eye expressions. Administration of a set of 28 photographs showing only the eye region of males and females, presented with one of four (1 target, 3 foil) words presented needs to be chosen that four best depict what the person may be feeling or thinking. Both cognitive and affective words are presented.

Note. [a]Hoogenhout et al., 2014 test battery; [b]Korkman et al., 2007; [c]Baron-Cohen, Wheelwright, Hill et al., 2001.

Figure 4.1. Examples of the stimuli presented in the child version of the "Reading the Mind in the Eyes" task (from Baron-Cohen et al., 2001). The word choices include both basic and more complex emotions and mental state words.

Cognitive ability. The WISC-IV (Wechlser, 2003) was used to assess IQ. A range of EF tasks were administered to investigate numerous EF domains. For details on both the IQ and EF measures see the *Methods* section in *Chapter Three* (pp. 44-49).

ADHD assessment. Each child was also assessed for ADHD. This diagnosis was based on a maternal interview administered by the longitudinal cohort's paediatrician using the K-SADS (Kaufman et al., 1997) and ratings by the child's classroom teacher on the DBD (Pelham et al., 1992). For details on the ADHD assessment see the *Methods* section in *Chapter Three* (pp. 50-51).

Procedure

For a detailed review of the general procedure, see the corresponding section in *Chapter Three* (pp. 52-53). Here I provide a brief summary only. Each child was tested individually on measures of ToM, IQ, and EF, while the mother was interviewed in a nearby room regarding demographic background and the child's school and health history. Testing took place over 2 days to avoid possible fatigue effects. Breakfast, a snack, and lunch were provided, and the child was encouraged to take breaks, as needed. Except in the most severely

affected FAS cases, examiners were blind regarding FASD diagnosis and alcohol exposure history.

Testing was conducted in Afrikaans or English, depending on the primary language of instruction in the child's school by two experienced Master's-level graduate research assistants. The ToM, WISC-IV, and EF tests were translated by a native Afrikaans-speaking Master's-level child psychologist with extensive experience working with the children in this cohort, and back-translated by the longitudinal cohort's paediatrician, a fluent Afrikaans speaker.

Human subjects approval was obtained from the Wayne State University (Appendix A) and University of Cape Town ethics committees (Appendix B). Informed consent was obtained from mothers at recruitment and child assessment visits (Appendix C and D); oral assent, from the children. Children received a small gift, and mothers received compensation consistent with guidelines from the ethics committees. For more detail on the general ethical considerations of the current research see the *Ethical considerations* section in *Chapter Three* (pp. 53-54).

Statistical Analyses

Statistical analyses of the behavioural data were conducted using the IBM Statistical Package for the Social Sciences (SPSS) version 22.0. The continuous alcohol and drug measures collected during pregnancy underwent log(x+1) transformation if they were skewed (skew>3.0) to reduce the effects of outliers. Four control variables were assessed— maternal education and smoking during pregnancy, and child sex and age at testing. The total raw score of each of the individual EF tests assessing cognitive flexibility and attentional control were averaged after transformation to z-scores. Only a single task was used for the assessment of goal setting and working memory and, thus, these domains were analysed using the respective

task's total raw score. Performance on the individual ToM tasks was measured by the allocation of a certain number of points given for each test question that was answered correctly. Correlations between the control variables and the ToM measures were calculated. Any control variable even weakly related to each ToM outcome ($p<.10$) was adjusted statistically in multivariate analyses of effects on that outcome. The relation of FASD diagnosis to the ToM outcome measures was examined using analysis of variance. Multiple regression was used to examine the relation between a continuous measure of PAE (AA/day during pregnancy) and each of the ToM outcome measures after control for potential confounders. Mediation was inferred if the entry of the mediator produced a statistically significant reduction in the raw regression coefficient for alcohol exposure, as assessed using the Sobel (1982) test for mediation (Soper, 2014). The relation of FASD and AA/day to ADHD was examined using ANOVA and Pearson correlation, respectively. The association of ADHD to ToM was examined using Pearson correlation.

Results

Sample Characteristics

Table 4.2 summarizes the demographic and background characteristics of the sample. The children came from a poorly educated, socioeconomically disadvantaged community. Mothers in the FAS/PFAS group were somewhat more economically disadvantaged (mean=Level V—Unskilled Labourers, lowest of five levels) than those in the HE and control groups (mean=Level IV—Semiskilled Workers) on the Hollingshead (2011) scale.

Table 4.2

Sample Characteristics

	FAS (*n*=8)	PFAS (*n*=19)	HE (*n*=17)	Controls (*n*=19)	*F* or χ^2	
Maternal age at delivery (years)	32.6 (6.9)	27.5 (7.2)	25.7 (4.9)	25.9 (3.4)	3.19	0.030
Education (years)	9.1 (2.2)	6.7 (2.6)	9.5 (2.0)	10.3 (1.3)	10.39	<0.001
Socioeconomic status[a]	16.2 (8.2)	15.2 (6.7)	23.7 (9.1)	27.4 (6.7)	9.70	<0.001
Alcohol use during pregnancy						
AA/day (oz)	1.8 (2.4)	1.0 (0.7)	0.5 (0.5)	0.0 (0.0)	8.49	<0.001
AA/occasion (oz)	4.7 (1.8)	3.8 (1.8)	3.0 (1.4)	0.1 (0.0)	34.17	<0.001
Frequency (days/week)	2.2 (1.9)	2.0 (1.0)	1.1 (0.8)	0.1 (0.0)	16.96	<0.001
Smoking during pregnancy (cigarettes/day)	8.7 (5.3)	7.7 (6.0)	7.5 (6.9)	1.9 (4.9)	4.63	0.006
Child characteristics						
Age at ToM and EF testing	10.6 (0.4)	11.1 (0.4)	11.1 (0.4)	11.0 (0.3)	3.60	0.018
Sex (% male)	37.5	57.9	58.8	47.4	1.42	0.702
WISC-IV IQ[b]	65.7 (8.2)	64.1 (10.2)	72.8 (7.6)	78.1 (10.5)	8.03	<0.001
ADHD[c] (number of cases)	1	7	2	3		

Note. Values are means (SD) or %. AA=absolute alcohol; 1 oz AA/day ≈ 2 standard drinks.
[a]Socioeconomic status (SES) at time of follow-up based on *Hollingshead* (2011) scale; mean for the FAS and PFAS = Level V (lowest of five social strata: Unskilled labor), while mean for the HE and control group = Level IV (semi-skilled workers).
[b]Wechsler Intelligence Scale for Children, Fourth edition.
[c]Met criteria for ADHD according to DSM-IV if (a) at least 6 of the 9 inattention and/or 6 of the 9 hyperactivity-impulsivity symptoms were endorsed ("pretty much" or "very much true") by one or more informants, and (b) some impairment was reported by 7 years in two or more settings.

As in previous studies (Jacobson et al., 2004; May & Gossage, 2011), mothers of children with FAS were older than those of other exposed children and controls. There was a significant between-group difference for both AA/day across pregnancy, AA/occasion and frequency (days/wk; all *p*s<.001), suggesting a dose-dependent relation between maternal drinking pattern and FASD diagnosis[1] (see Figure 4.2). Mothers of children with FAS and PFAS concentrated their drinking, resulting in consumption of an average of 3.6-4.8 oz AA/occasion (≈ 7.2 -9.6 standard drinks/occasion) on 1-2 days/week. All but one control mother (98.4%) abstained from drinking during pregnancy; the one control drank 2 drinks on three occasions. Mothers of exposed children also reported smoking more than controls during pregnancy. Drug use other than alcohol and smoking during pregnancy was rare across all participants: three women (4.8%) reported using marijuana (1-3 days/month), one used cocaine, and none used methaqualone ("mandrax"). Because prenatal exposure to these drugs was too rare for statistical adjustment, associations with prenatal alcohol use were rerun omitting the children exposed to these drugs.

Children with FAS were slightly younger than those in the other groups (p=.018), and there were no between-group differences in terms of sex distribution (Table 4.2). However, as in previous studies (see Vellante et al., 2013), non-exposed control girls performed more optimally than boys on the RME test (*M* for girls=17.6, *SD*=1.9, *M* for boys=16.7, *SD*=4.8, p=.001), a difference not seen in the alcohol-exposed children (p=.77). IQ differed significantly across the four groups: children with FAS had a lower IQ than controls (p<.01) but not than HE (p>.05); children with PFAS had a lower IQ than both HE (p<.01) and

[1] For all three continuous alcohol measures: FAS > HE, p<.01; FAS > Controls, p<.001; PFAS > HE, p<.05; PFAS > controls, p<.001; HE > Controls, p<0.01; for AA/day FAS > PFAS, p<.01 but there was no significant difference between the FAS and PFAS groups for AA/occasion (p>.05) and frequency of drinking (p=.433).

Controls (p<.001). IQ did not differ between the FAS and PFAS groups (p>.20) or between the HE and Control groups (p>.05).

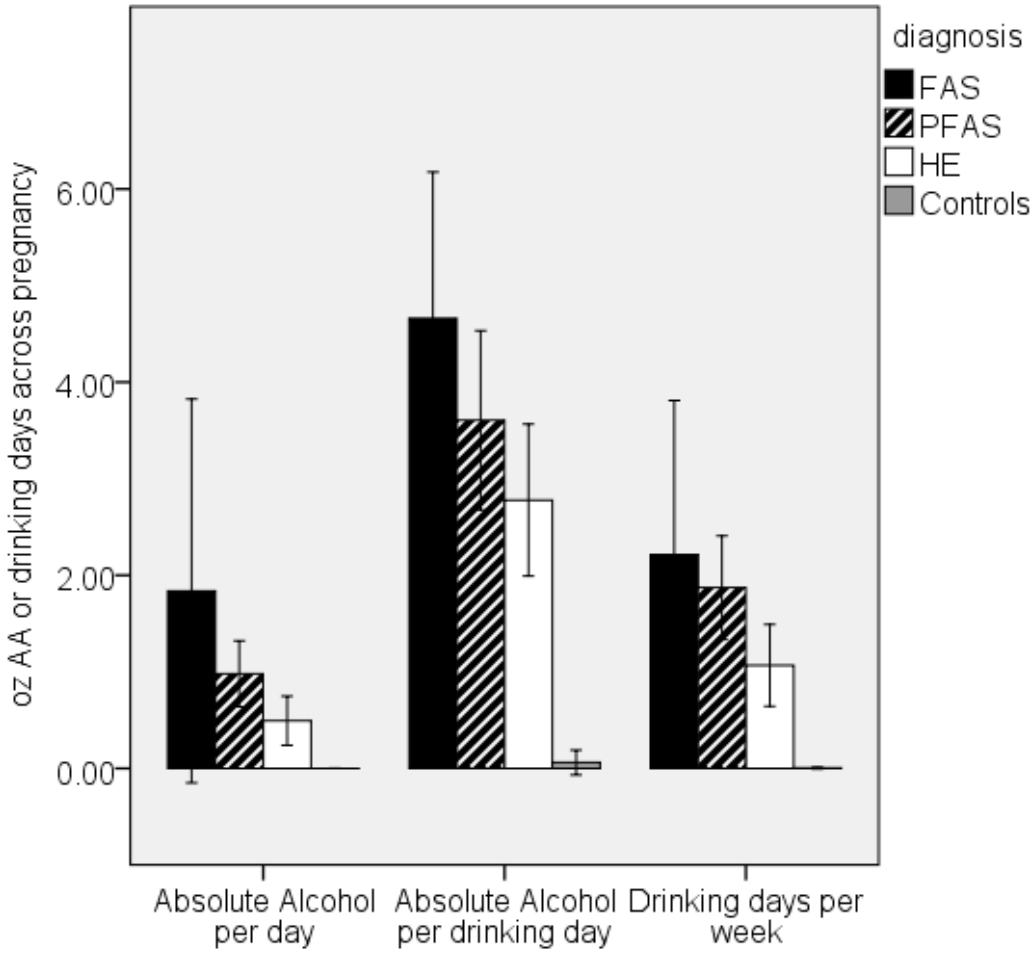

Figure 4.2. Relation of prenatal alcohol exposure to FASD diagnosis

ToM Findings

Potential confounders. Higher maternal education was associated with better performance on the First- and Second-order False Belief, Faux-Pas Detection and RME tasks (rs ranged from .30 - .47, all ps<.05; see Table 4.3). Socioeconomic status (SES) at recruitment and at the current follow-up visit, measured using the Hollingshead (2011) scale, was associated with the NEPSY-II AR and RME tasks (rs ranged from .23 – .53, all ps<.05). Maternal smoking during pregnancy was not related to any of the ToM measures. Boys

performed better than girls on NEPSY-II Contextual (p<.01). Child age at testing was not related to ToM performance, all ps>.10.

Table 4.3

Effects of Prenatal Alcohol Exposure and Potential Confounders on ToM Measures

		Absolute alcohol/day		Potential confounder	
Measures	*N*	*r*	ß[a]	*r*	ß[b]
Controlling for maternal education					
1st-order False Belief	61	-.10	0.12	.30**	0.37*
2nd-order False Belief	62	-.24*	-0.07	.34**	0.30*
Faux-Pas Detection	54	-.31**	-0.15	.36**	0.27†
Faux-Pas Comprehension	54	-.36**	-0.34*	.23*	0.04
Reading the Mind in the Eyes	63	-.55***	-0.42**	-.47***	-0.22†
Controlling for child sex					
NEPSY-II Contextual	62	-.31**	-0.32**	.23*	0.25*
Controlling for SES at recruitment					
NEPSY-II Affect Recognition	62	-.30*	-0.18	.34*	0.24
Reading the Mind in the Eyes	63	-.53***	-0.45***	.40***	0.17
Controlling for SES at follow-up					
NEPSY-II Affect Recognition	62	-.33**	-0.15	.37**	0.27†
Reading the Mind in the Eyes	63	-.53***	-0.33*	.53***	0.31*

Note. †p < 0.10; * p < 0.05; ** p < 0.01; *** p < 0.001.
Values are Pearson rs and standardised regression coefficients (ß).
[a]Effects of AA/day after control for potential confounder; [b]Effects of potential confounder after control for AA/day.

Relation of FASD to ToM. The analysis showed no between-group differences on the First- and Second-order False Belief, Strange Stories, and NEPSY-II tests (Table 4.4). Although the analyses detected no group differences in the ability to detect a faux pas, between-group differences in the ability to explain a faux-pas scenario (Faux-Pas Comprehension) fell just short of statistical significance (see Figure 4.3). Because of the trend that was seen on this task, I wanted to explore what would happen if the data for the three exposed groups were pooled together rather than having three separate alcohol-exposed groups. Comparing a PAE or FASD group rather than subtypes of FASD against a control group is very common in FASD research (see, e.g., Greenbaum et al., 2009; McGee et al., 2009; Rasmussen et al., 2013). The exposed children's scores on Faux-Pas Comprehension their scores were significantly lower than those of the controls, $t(52) = 2.68$, $p<.01$.

Analysis of the RME task showed a highly significant between-group difference even after controlling for maternal education, the only potential confounder, $F(3,57) = 7.54$, $p<.0001$ (see Table 4.4 and Figure 4.4). Children in the FAS and PFAS groups performed more poorly than those in the HE and control groups (FAS and PFAS<HE, both *p*s<.01; FAS and PFAS<controls, both *p*s<.001).

Table 4.4

Relation of FASD diagnosis to Theory of Mind measures

FASD diagnostic group	FAS	PFAS	HE	Controls	*F*	*p*
1st-order False Belief *(Max raw score = 12)*	11.4 (1.5)	11.3 (2.4)	12.0 (0.0)	11.0 (2.9)	0.67	.574
2nd-order False Belief *(Max raw score = 8)*	2.0 (2.9)	2.9 (2.0)	3.8 (2.1)	3.9 (2.0)	2.02	.121
Strange Stories *(Max raw score = 48)*	25.6 (6.3)	29.5 (5.9)	30.2 (5.3)	29.9 (6.1)	1.11	.354
Faux-Pas Detection *(Max raw score = 5)*	2.1 (1.4)	2.0 (1.5)	2.7 (1.7)	2.8 (1.9)	2.57	.506
Faux-Pas Comprehension *(Max raw score = 5)*	1.4 (1.6)	1.7 (2.4)	2.4 (2.6)	4.2 (4.0)	2.57	.065
NEPSY-II Contextual *(Max raw score = 6)*	3.5 (1.2)	4.1 (1.2)	4.3 (1.3)	4.4 (0.9)	1.24	.303
NEPSY-II Affect Recognition *(Max raw score = 35)*	22.6 (2.7)	22.9 (5.5)	24.1 (2.6)	24.2 (3.2)	1.07	.370
Reading the Mind in the Eyes *(Max raw score = 28)*	10.0 (2.4)	12.2 (2.8)	15.4 (4.6)	17.2 (3.5)	10.98	<.001

Note. Values are means (SD) of raw scores.
1st-order False Belief (FAS: *n*=7; PFAS: *n*=19; HE: *n*=17; Controls: *n*=18)
2nd-order False Belief (FAS: *n*=8; PFAS: *n*=18; HE: *n*=17; Controls: *n*=19)
Strange Stories (FAS: *n*=7; PFAS: *n*=13; HE: *n*=13; Controls: *n*=17)
Faux-Pas Detection (FAS: *n*=7; PFAS: *n*=15; HE: *n*=15; Controls: *n*=17)
Fax-Pas Comprehension FAS: *n*=7; PFAS: *n*=15; HE: *n*=15; Controls: *n*=17)
NEPSY-II Contextual (FAS: *n*=8; PFAS: *n*=19; HE: *n*=16; Controls: *n*=19)
NEPSY-II Affect Recognition (FAS: *n*=8; PFAS: *n*=19; HE: *n*=16; Controls: *n*=19)
Reading the Mind in the Eyes (FAS: *n*=8; PFAS: *n*=19; HE: *n*=17; Controls: *n*=19)

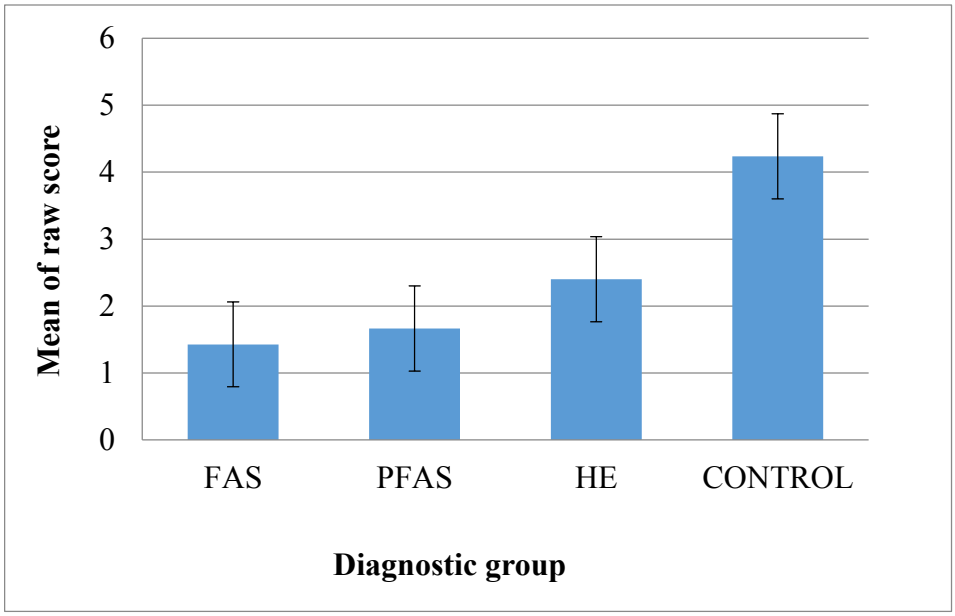

Figure 4.3. Relation of FASD diagnostic group to the Faux Pas Comprehension task (showing a trend toward statistical significance; p=.065)

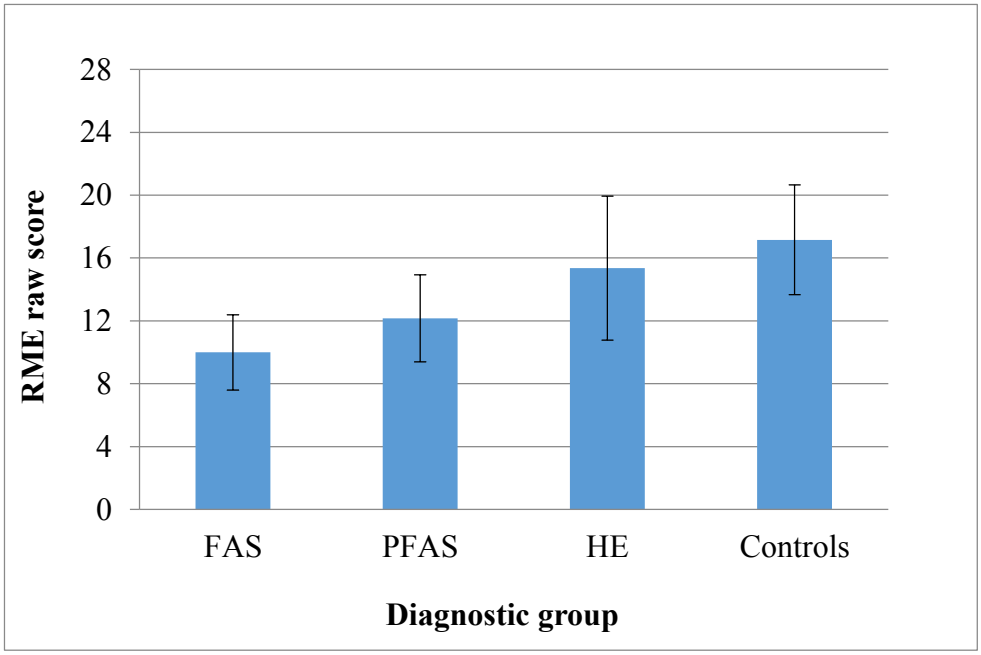

Figure 4.4. Relation of FASD diagnosis to performance on the Reading the Mind in the Eyes task; Maximum raw score for RME is 28.

Relation of AA/day to ToM. AA/day was related to four of the ToM measures (Faux-Pas Comprehension, NEPSY-II Contextual, NEPSY-II AR, and RME) after control for potential confounders (Table 4.3), with the magnitude of the effect on the RME task clearly the strongest. The associations of PAE with Second-order False Belief and Faux-Pas Detection were accounted for by variation in maternal education. By contrast, the effect on Faux-Pas Comprehension remained significant after control for maternal education, as did the effect on RME. Similarly, the alcohol effect on NEPSY-II Contextual remained significant after adjustment for child sex. Although the relation of alcohol on the NEPSY-II AR was no longer significant after adjustment for SES at recruitment and follow-up, the relation of alcohol exposure to RME remained significant after control for SES.

The NEPSY-II AR task uses two different formats of stimulus presentation: (1) facial photos for each problem presented simultaneously; (2) stimuli to be compared presented sequentially, thus entailing an element of WM. Simple facial AR, measured by the simultaneous items, was not altered by PAE at this age (r=-.09, p>.20). By contrast, the effect of PAE on the more challenging sequential AR items (r=-.43, p=.001) remained significant after control for WM as measured by the 1-back (β=-0.47, p=.012) and 2-back (β=-0.42, p<.05) tasks. Thus, the alcohol effect on the sequential AR items may not have been attributable to impaired WM ability

Mediation by IQ and EF. Regression analyses relating AA/day and IQ to each of the ToM measures are shown in Table 4.5. Results of the Sobel tests indicate that the effects of PAE on Faux-Pas Comprehension and NEPSY-II AR were significantly mediated by IQ, and were no longer significant after adjustment for IQ, indicating that alcohol-related IQ deficits play a major role in the observed ToM impairment in these domains. By contrast, the effect of AA/day on RME was only partially mediated by IQ and continued to be significant after

adjustment for IQ, indicating that PAE impacts RME over and above its effect on overall intellectual function. IQ did not significantly mediate the effect of prenatal alcohol on performance on the NEPSY Contextual task.

Table 4.5

Mediation of the Effects of Prenatal Alcohol Exposure on ToM Measures by IQ

Measures	*N*	*r*	ß	Sobel *z*
1st-order False Belief	61	-.10	-0.04	-0.76
2nd-order False Belief	62	-.23†	-0.02	-2.55**
Strange Stories	50	-.17	0.04	-3.16**
Faux-Pas Detection	54	-.29*	-0.11	-2.36*
Faux-Pas Comprehension	54	-.32*	-0.14	-2.45**
NEPSY-II Contextual	62	-.31**	-0.21	-1.29
NEPSY-II Affect Recognition	60	-.33**	-0.12	-2.41*
Reading the Mind in the Eyes	63	-.54***	-0.29**	-3.18**

†$p < .1$; * $p < .05$; ** $p < .01$; *** $p < .001$.
r shows the relation between AA/day and each of the ToM measures.
ß is the standardised regression coefficient relating AA/day to the ToM measure after statistical adjustment for WISC-IV IQ.

In contrast to the IQ-related effects, there was little mediation of the effects of PAE on the ToM measures by EF (Table 4.6). The alcohol effect on Faux-Pas Comprehension was mediated by cognitive flexibility, Sobel z=-2.45, p=.014, and mediation of the effect on Faux-Pas Detection by WM fell short of statistical significance, Sobel z=1.89, p=.058. None of the EF measures mediated any of the other effects of PAE on ToM measures, all ps>.15. WM had a much stronger effect on Faux-Pas Detection than on Faux-Pas Comprehension, suggesting

that it specifically impacted the child's ability to hold the story in memory and then to manipulate it. The effect of PAE on RME remained significant even after controlling simultaneously for IQ and all EF measures for both AA/day (β=-0.28, $p<.05$) and FASD diagnosis ($F(3,53) = 4.90$, $p<.01$.).

Table 4.6

Effect of Prenatal Alcohol Exposure on ToM Performance Controlling for Executive Function

			Mediating variables			
			Working memory	Goal setting	Attentional control[a]	Cognitive flexibility
Measures	*N*	*r*	ß	ß	ß	ß
1st-order False Belief	61	-.10	0.01	-0.10	-0.18	-0.06
2nd-order False Belief	62	-.23†	-0.18	-0.23†	-0.26†	-0.21
Strange Stories	50	-.17	-0.11	-0.16	-0.12	-0.15
Faux-Pas Detection	54	-.29*	-0.16†	-0.29*	-0.31*	-0.23
Faux-Pas Comprehension	54	-.32*	-0.24	-0.32*	-0.36*	-0.26†
NEPSY-II Contextual	62	-.31*	-0.29*	-0.30*	-0.29*	-0.31*
NEPSY-II Affect Recognition	60	-.33**	-0.29*	-0.32**	-0.31*	-0.28*
Reading the Mind in the Eyes	63	-.54***	-0.48**	-0.54***	-0.51***	-0.50***

Note. †$p < .1$; * $p < .05$; ** $p < .01$; *** $p < .001$.
r shows the relation between AA/day and each of the ToM measures. ß is the standardised regression coefficient relating AA/day to the ToM measure after statistical adjustment for executive function mediating variables.
[a]Estimated for 6 cases

Controlling for drug use. Because very few mothers reported using drugs, I reran the effect of PAE on the RME task excluding data of the cases that reported drug use. The effect of alcohol on RME remained significant when analysed in relation both to diagnostic groups, $F(3,54) = 9.86$, $p<.001$, and to the continuous alcohol measure, $r=-.54$, $p<.001$.

PAE and ADHD. ADHD was significantly related to RME ($r=-.25$, $p=.047$), not significantly related to First-order False Belief ($r=-.23$, $p=.071$), and not related to any of the other ToM measures (all ps$>.15$). There was an adverse effect on RME of number of Inattentive ($r=-.31$, $p=.014$), but not Hyperactive-Impulsive ($r=-.15$, $p=.255$), symptoms of ADHD. Multivariate analyses showed that FASD diagnosis ($F(3,58) = 10.46$, $p<.001$) and AA/day ($\beta=-0.51$, $p=.001$) were each significantly related to RME after control for ADHD, whereas ADHD was no longer significantly related to RME ($\beta=-0.14$, $p=.20$) after control for alcohol exposure. Similarly, regression analyses showed that prenatal alcohol ($\beta=-0.54$, $p=.001$) was independently related to the RME, whereas Inattentive ($\beta=-0.13$, $p=.25$) and Hyperactive symptoms were not ($\beta=-0.07$, $p=.559$).

Discussion

Although children with FASD have been described as having alcohol-related social problems, the potential of an environmental insult, such as PAE, to alter ToM function has received little attention. In typically developing children, ToM ability only reaches certain milestones by the age of 11. Therefore, assessing children with FASD at this age, when basic ToM abilities should be fairly well developed, may help demonstrate whether impairment is present when compared to healthy controls of the same age.

Despite cross-cultural variations, children understand First- and Second-order False Belief by preschool and early school age, respectively. There were no group differences on First- and Second-order False Belief tasks within the current sample, suggesting that the

ability to infer epistemic mental states at both First- and Second-order levels was equally developed across all groups at age 9-11 years. Previous research on First-order False Belief in FASD found significant impairment in younger alcohol-exposed children (Rasmussen et al., 2009). The findings of the current research suggest that the deficit in this elementary form of ToM is no longer evident in older school-age children, which in turn suggests that the earlier deficit was attributable to developmental delay rather than arrested development. The current findings are also consistent with findings from another study of school-aged children with FASD in which no differences were found on Second-order False-Belief tasks (Greenbaum et al., 2009).

The most robust findings involved the effects of PAE on RME task performance. These effects were seen when the data were analyzed using FASD diagnostic group as the predictor and when using the continuous measure of PAE as the predictor. Together, these findings suggest that alcohol-exposed children have deficits in the attribution of simple and more complex mental states, a critical aspect of ToM. Specifically, the impairment is evident when required to infer mental states of others via expressions portrayed through the eyes. The results demonstrated here are consisted with the idea that prenatal alcohol exposure effects RME and that this association was not accounted for by deficits in IQ or EF or by ADHD, or by sociodemographic background.

This deficit is likely most clearly evident, in part, because RME is the ToM task that is least dependent on general cognitive function. Baron-Cohen et al. (1997) characterize it as a "pure" ToM task as it clearly does not require engagement of EF domains, such as attentional control. In contrast to Faux-Pas Comprehension, the RME task also does not require cognitive flexibility. It makes only minimal demands on WM because a written version of the affective words and mental state phrases are left in front of the child. Also, knowledge of the meaning

of the words is not essential as participants are encouraged to ask the meaning of any unfamiliar words. Furthermore, there is no time limit. Thus, the RME data indicate that impaired responsiveness to emotional cues in children with FASD cannot be attributed to poor attention or executive dysfunction but are reflective of the notion that PAE is specifically related to deficits in affective processing necessary for interpretation of complex mental states. These data are consistent with Kerns et al.'s (2016) recent findings that children with FASD have more difficulty with emotion recognition than controls.

I demonstrated that the association between PAE and poor RME persists after adjustment for IQ, which is consistent with previous findings in which no significant association was found between RME performance and IQ in adults (Baron-Cohen, Wheelwright, Hill et al., 2001). In the current study, the RME effects also persisted after adjustment for EF. Previous studies have found activation in the amygdala, the superior temporal gyrus and the posterior superior temporal sulcus, using RME or other affective processing tasks (Adams et al., 2009; Barbour et al., 2010; Baron-Cohen, Ring et al., 1999). Hence, in future neuroimaging research one could expect alcohol-related deficits in brain regions related to affective processing in children with FASD.

Investigation of higher-order ToM abilities revealed that alcohol-exposed and control participants performed equally well on the Strange Stories and Faux-Pas Detection tasks. These findings suggest that the ToM deficits in FASD are less severe than those seen in children with ASD, in whom deficits on Strange Stories and Faux-Pas continue to be detected at school age (Baron-Cohen, O'Riordan et al., 1999; Happé, 1994). However, a between-group difference was shown on Faux-Pas Comprehension when comparing all individuals with PAE, independent of FASD diagnosis, to control children. This finding supports the idea that PAE is indeed related to deficits in understanding a Faux-Pas, and may adversely affect

the various evaluation processes (as outlined in the SIP model) during social interactions. In other words, individuals with heavy PAE are able to recognize a social faux pas (as indicated on their performance on the Faux-Pas detection task) but demonstrate difficulties in correctly interpreting them. This piece of data is in contrast to recent findings from studies examining faux pas in adults (aged 18-41 yrs) using a similar story-based faux-pas task where it was shown that in comparison to healthy controls, individuals with FAS had difficulties identifying a faux pas (Rangmar, Dahlgren, Sandberg, Aronson, & Fahlke, 2015). However, the authors did not include a measure that would have indicated whether participants, rather than simply identifying a faux-pas as being present, were able to also correctly understand the faux-pas, which was the case in the current Faux-Pas Comprehension variable.

Furthermore, the current data are also consistent with the idea that other higher-order ToM domains are adversely affected by PAE at school age. Given that EF might be expected to play an important role in social information processing, it is striking that virtually none of these effects were mediated by EF. Performance on two of the four affected ToM tasks—Faux-Pas Comprehension and NEPSY-II AR—were mediated by PAE effects on IQ. Dependence of these more complex tasks on higher-order cognitive function makes it difficult to determine whether the observed effects are attributable to the alcohol-related IQ effects or are merely more difficult to detect after adjustment for IQ. However, the findings that fetal alcohol-related ToM deficits on the NEPSY-II Contextual and RME tasks were not accounted for by IQ support the idea that PAE is independently related to specific higher-order ToM impairments in FASD.

The previous FASD study that used the NEPSY-II ToM assessment, which combines Verbal and Contextual tasks, found no alcohol-related impairment (Rasmussen et al., 2013). In the current study I only administered the Contextual task from the NEPSY-II ToM

assessment because the Verbal component provides a broad assessment of social cognition and is not specifically focused on ToM. In the Contextual task, the child cannot see the facial expression but must infer what the person might be feeling given the context. As noted earlier, these data suggest that the effect of PAE on performance on the NEPSY-II Contextual task was not mediated by IQ or EF, and was not attributable to potential confounders. Thus, this task and RME, which both assess mental state attribution, appear to be sensitive to PAE.

I evaluated performance on the ToM tasks using both FASD diagnosis and a measure of continuous alcohol exposure as a predictor. Although the FASD diagnostic groups clearly differed in RME performance, when evaluating performance on the individual ToM tasks using a continuous measure of alcohol exposure instead, differences on more tasks were revealed related to amount and timing of alcohol exposure rather than a diagnosis along the fetal alcohol spectrum. The continuous alcohol measure used in the analysis measured prenatal maternal alcohol consumption as absolute alcohol per day throughout the pregnancy and was more sensitive than FASD diagnosis in detecting alcohol-related deficits. In this analysis, three other ToM tasks (Faux-Pas Comprehension, NEPSY-II Contextual and NEPSY-II AR) were shown to have differences related to the pattern of maternal drinking, when performance was not evaluated in realtion to FASD diagnosis. Except for on NEPSY-II AR, between-group differences remained significant even after adjustment for potential confounders. Thus, performance on the significant tasks suggests that impairments in the specific ToM ability examined are related to the pattern of PAE, and not to individual variations in demographic background. Also, the fact that performance on the NEPSY-II AR was confounded by level of SES and mediated by the effect of alcohol on IQ, suggests that future research should use a different task to investigate affect recognition in children with FASD.

Limitations

This study has limitations common to other longitudinal studies of PAE in that it depends on the mother's report of her alcohol consumption during pregnancy. A strength of this study, however, is that PAE was determined prospectively on the basis of maternal reports obtained during pregnancy. Moreover, the alcohol ascertainment protocol used has been validated in relation to levels of fatty acid ethyl esters in meconium samples in this community (Bearer et al., 2003) and in relation to child cognitive outcomes (e.g., Jacobson et al., 2002; 2004).

Furthermore, in previous studies examining ToM in individuals with PAE, group comparisons were made between a FASD group and controls, without considering the possible unique deficits that may manifest themselves within each diagnostic subtype. Instead, in the current study performance of three diagnostic groups was compared to a control group recruited simultaneously during the retrospective recruitment process of the PAE participants. Additionally, performance was also investigated using a continuous alcohol measure, independent of diagnosis.

It is important to consider the unique aspects of the cohort when considering generalising these findings. The binge-like pattern of maternal drinking, for example, is typical for individuals in the Western Cape but may be very different to other populations. Also, the socio-economic environment and circumstances to which the children in this cohort were exposed, limit their access to basic services. In addition, poor nutrition, which is common in the community from which these children were recruited, may also be an aspect specific to this cohort. However, an important strength of the current study is its sample size. In addition, all of the participants were recruited from the Cape Coloured community. Many communities with a similar demographic background exist throughout the Western Cape, and

not only in the location where the current cohort was recruited from. Thus, these findings can be extended to other FASD populations within South Africa but, moreover, also warrant replication in other populations.

Unlike a real world setting, the stimuli used in the RME are static and only resemble the cognitive processes involved in a natural social interaction. However, the RME is a valid and 'pure' ToM task that is quick and easy to administer to both adults and children (Baron-Cohen, Wheelwright, Hill et al., 2001; Baron-Cohen, Wheelwright, Scahill et al., 2001). In this study, I demonstrated that the effect of alcohol on RME persists even after adjustment for IQ and EF, which is consistent with previous findings in which no significant association was found between RME performance and IQ in adults (Baron-Cohen, Wheelwright, Hill et al., 2001). These findings extend this previous work by showing the RME to be a useful and valid test for detecting subtle impairment in social cognition independent of IQ and EF in children with FASD.

The large number of comparisons made during statistical analysis in this study increased the probability of making a Type 1 error. However, because this is the first study to examine ToM in such detail using a developmentally sensitive battery, I was prepared to risk a Type 1 error in the interest of exploring the data thoroughly. Furthermore, in trying to reduce the probability of this error, significant group differences were only further considered if the size of the effect was at least medium.

Conclusions and Future Directions

The current findings suggest that deficits in higher-order ToM function, specifically mentalizing through eye expression, may play a significant role in the behavioural impairment often described clinically in children with FASD. People's eyes are thought to provide us with invaluable social cues as they allow us to decode other's intentions; hence, they are a crucial

guide for our social interactions. Children with FASD have difficulty reading people's mental states, and this deficit may contribute to their deficits in social cognition.

Within the theoretical frameworks guiding this research project, the initial aim was to examine whether there is an alcohol-related effect on the interpretation of social cues in children with FASD. The rationale presented suggested that if such a deficit was present, examining the precursor to interpreting facial social cues, i.e., correctly identifying facial emotions through intact emotion recognition ability, would help to clarify whether a deficit at the level of interpretation was secondary to deficits at the level of emotion recognition. In this study, the highly significant findings of the RME task were consistent with the idea that an alcohol-related deficit does indeed exist at the interpretation level, specifically suggesting an impairment in the ability to infer other's mental states in children with FASD. Moreover, in the current study, performance on the NEPSY-II AR task, which uses both simultaneous and sequential face stimuli presentation to assess emotion recognition ability, was shown to be significantly related to PAE. Differences were shown specifically on the sequential AR component, i.e., when a WM component was part of the task. Thus, in line with the theoretical frameworks, future research should aim to examine emotion recognition ability in these children as an antecedent to the higher-order social-cognitive deficits observed here.

Correlations with teacher and parent report measures may provide valuable insight into whether the deficits as measured by the RME translate into problematic behavioural outcomes. Also, if it is true that alcohol-exposed children have difficulties inferring the mental states of others, interventions using developmentally appropriate tasks may help alcohol-exposed children focus on and improve their ability to read affective cues and facial expressions. Such training may help overcome some of these deficits in social attention or affective information processing. However, prior to establishing an intervention protocol,

further research into the potentially altered mechanisms underlying the processing of affective face stimuli is required.

CHAPTER FIVE: Study II - Affective appraisal and working memory in children with fetal alcohol spectrum disorders

Introduction

The irreversible cognitive and neurodevelopmental deficits following heavy PAE in individuals with FASD are well established. Much research has focused on characterizing the various domains of social deficits that may manifest themselves in, for example, social skills problems (Thomas et al., 1998) that individuals with FASD are known to have. Very few studies, on the other hand, have considered the underlying cognitive mechanisms responsible for FASD-specific social deficits.

In Study I of this dissertation, I investigated a broad range of ToM abilities in children with FASD. The most significant finding was that children with FAS and PFAS made more errors compared to HE nonsyndromal and control children on the child version of the RME test. In addition, PAE was associated with poorer performance on the RME test. In this task, the participant is asked to choose one descriptor from four stimuli words (1 target, 3 foil) that best matches the mental state portrayed in eye expressions (see Figure 4.1, pg. 76). The RME task was the only test from the comprehensive ToM test battery, in which the effect of alcohol was still significant after controlling for potential confounding variables (e.g., sex, age at testing, SES and maternal smoking and education) as well as mediators (e.g., child IQ and executive function. These findings suggest that children with FASD have difficulty reading people's emotions and social cues from facial expressions.

Recognition of facial expression is a crucial step in both the SIP and ASC models reviewed in *Chapter Two*. I was, therefore, interested in further investigating these children's emotion recognition ability based on the strong findings of the RME test.

Despite the occurrence of social problems in individuals with PAE (e.g., Carmichael Olsen et al., 1997; Jacobson, Jacobson et al., 2006; McGee et al., 2009; Rasmussen et al., 2013) and the little research on PAE-related ToM impairments, there is also only limited research examining social-emotional functioning in FASD. A literature search yielded only one study (Greenbaum et al., 2009) that specifically investigated emotion processing in children with FASD. This study compared social-cognitive performance of 6- to 13-year-old children with FASD (mean age = 9.2 years) and ADHD (mean age = 9.3) in relation to a healthy control group (mean age = 8.9). In addition to other cognitive measures, the study examined emotion processing using four subtests of the Minnesota Test of Affective Processing (MNTAP; Lai, Hughes, & Shapiro, 1991). Children in the FASD group performed more poorly than those in the ADHD and healthy control groups on only one of the MNTAP subtests. This subtest, assessing Affect Choice, requires matching a verbally generated emotion to one of a series of faces displaying different emotions. Specifically, a photograph is shown of a person with five different emotional expressions, and the participant has to touch the face on the computer screen that depicts the emotion that is verbally generated by the computer. Hence, this test assesses the ability to correctly identify facial expressions. Unlike the Affect Choice subtest, the other MNTAP subtests, which showed no significant group differences, could all be solved perceptually without understanding the actual emotion in question. Moreover, these social cognition and emotion processing data were associated with teacher reports indicating that difficulties in this domain were linked to behavioural and social problems. Emotion processing specifically, and not social cognition, was shown to be related to impairments in social skills. Emotion processing may, therefore, contribute to this population's social behavioural problems. Of note here is that the most significant finding in the social-cognitive battery was seen on a task that required the correct

identification of facial affect. Similarly, the RME test requires the recognition of mental states including basic emotions, such as 'happy' and 'sad', from the eye regions of the face.

The assessment of facial affect recognition in Study I, found no significant between-group differences on the NEPSY-II AR subtest (Korkman et al., 2007) in relation to FASD diagnosis. However, when looking at the descriptive statistics associated with performance on that task, on average FAS (M=22.6, SD=2.7) participants performed somewhat worse than the PFAS group (M=23.0, SD=2.6), who scored lower than HE groups (M=24.1, SD=2.6), who, in turn, performed more poorly than Controls (M=24.2, SD=3.2), although not significantly so. A comparison of all the FASD participants with the control group found no significant between-group differences, $t(60)$=-1.113, p=.270. These results are consistent with the findings from the only other study in which the NEPSY-II AR task was administered to children with FASD (Rasmussen, 2013). By contrast, in Study I there was a significant effect of PAE for performance on the AR task when a continuous measure of alcohol exposure was used, suggesting that this alcohol exposure variable was more sensitive than when analyzing performance by diagnostic group (see *Chapter Four,* p. 86).

Although subsequent analyses showed that the effect of PAE on performance on the NEPSY-II AR task was mediated by IQ, I further wanted to investigate affect recognition in children with FASD. For this purpose, in this second study, an affective appraisal task was chosen aimed at minimizing the impact of IQ and other potential mediators or confounders.

In the context of the selected task administered in Study II, the term 'affective appraisal' refers to the judgment a participant makes about a displayed facial emotion. The current task is a continuous affective performance task during which a participant appraises whether an affect signaled by a face was the same or different to the affect signaled by a face on the previous trial (Barbour et al., 2010). The underlying rationale for employing this task

to investigate social-cognitive problems in children with FASD is the notion that if a child shows impairments in affective appraisal this may impact their ability to read facial social cues, which in turn influences their social behaviour and interactions. This task solely consists of stimuli in the form of sequential photographs of people's faces and features no verbal or written items. Responses are made by pressing one of two buttons on a keyboard. Hence, it was anticipated that performance on this task would be only minimally affected by IQ.

However, the design of the selected task may place demands on WM. Because WM has been shown to be impaired in alcohol-exposed children (Burden et al., 2005; Diwadkar et al., 2011) its involvement in the task may adversely influence performance. It was, therefore, also important to evaluate the degree to which WM may impact on the appraisal of affective faces in the current task. Importantly however, in a real-life setting, affective appraisal during social interaction also requires WM input and thus, the design of this task may indeed be ecologically valid.

Working Memory and FASD

WM is a cognitive system that provides temporary storage of information that needs to be manipulated before it can be utilised in other complex cognitive processes, such as language comprehension (Baddeley, 1992). WM is crucial for the mental manipulation of information and thus essential for engaging in cognitive tasks. WM is considered an integral component of EF as it is a basic mechanism crucial for many other EF processes, such as attention and planning (Burden et al., 2005; Diwadkar et al., 2012; Jacobson & Jacobson, 1999; Kodituwakku et al., 1995; O'Malley & Nanson, 2002; Rasmussen, 2005). Several studies have shown that PAE is related to deficits in WM (specifically, poor performance on the WISC Digit Span test; Carmichael Olson, Felman et al., 1998; Jacobson, Jacobson, Sokol, Chiodo et al., 1998; Streissguth et al., 1990). Furthermore, WM deficits following PAE

persist even when general intellectual ability (IQ) is adjusted statistically (Burden et al., 2005).

Hence, the impact of WM on overall task performance is an important cognitive domain to consider when conducting FASD research. Streissguth et al. (1990) proposed that social problems are secondary to attention and EF problems, including WM. By contrast, Jacobson, Jacobson, Sokol, Chiodo et al. (1998) have shown that alcohol-related social problems reported by teachers on the Achenbach (1991) Teacher Report Form in their Detroit Longitudinal Alcohol Cohort were primary deficits of prenatal exposure and not secondary to attentional problems.

Given the known deficits in WM related to PAE and the design of the affective appraisal task, the potential effect of WM ability on affective appraisal ability as measured by the current task, needed to be established. For this purpose, WM ability was concurrently assessed in this study using a second measure.

Study II Aims and Hypotheses

This is, to my knowledge, the first study to investigate affective appraisal in children with FASD. It builds on evidence that these children have deficits in emotional information conveyed via facial expressions (Study I). The current study aimed to examine (1) WM ability at different levels of complexity for each of the three FASD diagnostic groups compared to typically developing controls; (2) how WM ability impacts on the affective appraisal task; (3) whether there are between-group differences in affective appraisal ability after adjustment for WM; and (4) whether there is an PAE-related effect on both WM and affective appraisal independent of FASD diagnosis.

I hypothesized that children with FASD would perform more poorly on the WM task compared to healthy controls and that difficulties would be associated to the task's

complexity level. Specifically, I predicted that children with FAS would perform more poorly than children with PFAS, who would perform worse than the HE children, who would perform worse than typically developing children. Furthermore, I hypothesized that an effect would also be seen independent of FASD diagnosis when considering a continuous measure of PAE. I further hypothesized that children with FASD, nonetheless, demonstrate sufficient WM capacity to engage in the affective appraisal task. Lastly, for performance on the affective appraisal task, I expected to see between-group differences and a PAE-related effect (i.e., independent of FASD diagnosis).

Methods

Participants

I administered a WM and an affective appraisal test to a sample of 88 Cape Coloured (mixed ancestry) children aged 9 to 12 years. Participants were recruited from the Cape Town Longitudinal Cohort described in *Chapter Three* (see p. 40).

Procedure

The measures described below were administered as part of a larger neuropsychological test battery over a 2-day visit to the CDRL. On Day 1, participants completed both levels of the WM tasks with a break in-between. On Day 2, the affective appraisal task was administered. For details on the general testing procedure and ethical considerations, see the *Procedure* section in *Chapter Three* (see pp. 52-54).

Measures

Both WM and affective appraisal were assessed using an *n*-back and are described in detail below. Furthermore, to examine the potential mediation of IQ and EF on the effect of alcohol on both affective appraisal and WM, the data from the WISC-IQ (Wechsler, 2003)

and various EF measures collected during Study I, were also analysed in this study. For more details on the IQ and EF tasks, see the *Measures* section in *Chapter Three* (pp. 44-49).

Working memory task. An experimental paradigm known as the *n*-back task was used to assess WM ability in the participants. The *n*-back is frequently used to assess WM in experimental settings (e.g., Barbour et al., 2010; Diwadkar et al., 2013; Owen, McMillan, Laird, & Bullmore, 2005). During this computer-based task, the child is required to identify whether a visual stimulus (e.g., a single letter) currently presented is the same or different from the stimulus presented *n* trials before. In this study, I used an n-back assessment adapted from Casey et al. (1995). I administered a 1-back version, where the target stimulus is evaluated with reference to the stimulus presented immediately before the target stimulus; and a 2-back version, where the target stimulus is evaluated with reference to the stimulus presented two items before the target stimuli.

The examiner described the task to the child, who was seated in front of a computer. The examiner demonstrated that a series of letters would be displayed on the screen one at a time, and that each time a new letter came up on the screen, the participant needed to decide whether the letter currently displayed is the same or a different letter from the previous one (1-back). A paper practice trial was used to test the child's understanding of these instructions. Further instructions regarding pressing the response buttons followed. The participant was informed that once s/he had decided whether the letter on the screen is the same or different from the previous letter, s/he had to press a specific key on the keyboard (same), or another designated key (different). The two target keys were marked with stickers to distinguish them from the other keys on the keyboard and to minimize buttons other than the target buttons from being pressed. A second paper practice trial was then run in which the new instruction of pressing the key for "same" and the key for "different" was incorporated.

Following the practice round, the correct knowledge of the keys was assessed by asking the participant to demonstrate which key is pressed when.

By giving consecutive verbal responses on the paper practice trial, the participant demonstrated that s/he understood that a response was required (i.e., a key had to be pressed) following each stimulus. Then, a practice computer trial was run simulating the actual 1-back computer task. On successful completion of the computer practice trial, the actual 1-back task was administered for four consecutive trials of 12-letter blocks. Each letter appeared on the screen for 500ms, followed by a 2500ms inter-stimulus interval. A fixation cross was presented after each block of trials for 15s.

On successful completion of the 1-back test, a non-computer based cognitive test from the larger Neuropsychological test battery was administered to give the child a before explaining the 2-back version. The 2-back test was only administered to individuals who successfully completed the 1-back task (i.e., when participant's responses were not random but reflective of the task rules). Similarly to the 1-back assessment, instructions for the 2-back were given and the understanding thereof tested with two paper practice trials and one computer-based practice trial before administering the actual 2-back task. In the instructions for the 2-back, the participant was told that s/he had to decide whether the letter on the screen was the same or different to the one presented two letters before. On successful completion of the computer practise trial, the actual 2-back task was run over four consecutive trials of 12-letter blocks. The *n*-back tasks were programmed and run using E-Prime software (Psychology Software Tools, Inc., Pittsburgh, USA) and responses to the stimuli were recorded on the computer.

The affective appraisal task. The affective appraisal paradigm chosen for this study has been successfully employed in neuroimaging research with children and adolescents at risk

for schizophrenia (e.g. Barbour et al., 2010; Diwadkar et al., 2012). The task uses a *n*-back format, in which the participant decides whether the face displayed on a screen shows the same or a different affect as the face shown previously (see Figure 5.1). Generally, affective *n*-back paradigms are designed to assess the implicit processing mechanism when exposed to affective stimuli. The paradigm used in the current task, however, aimed instead at assessing the explicit valence of the affective stimuli itself, without the attachment of affective labelling and independent of face identity (Diwadkar et al., 2012). Affective appraisal of faces in a WM task provides an appropriate opportunity to examine relations between both the cognitive and social deficits seen in children with FASD.

As in the 1-back test described above, the participant was presented with continuous stimuli presented sequentially on a computer screen. The affective appraisal task was programmed and run using E-Prime software by Diwadkar (2012). The picture stimuli used are grey-scale pictures of people's faces (male and female) using normatively rated photographs (Ekman & Oster, 1979) portraying different basic emotions across three differently valenced emotion categories, namely positive (happy), negative (angry, fearful, and sad) and neutral.

The affective appraisal task uses only basic emotions. The more complex secondary emotions, such as pride, guilt and embarrassment, are acquired through learning and experience and are uniquely human (Panksepp, 1998). However, secondary emotions are social, self-conscious emotions, which are extensively internalized and are triggered as a response to cognitive self-evaluations. They are not associated with any characteristic facial expression, unless co-occurring with a more basic emotion. Secondary emotions are, therefore, not suited for the investigation of affective appraisal using face stimuli. The

affective appraisal task does not require explicit labelling of the displayed affect but simply a judgment-type comparison without labelling the actual affect displayed.

A total of 80 faces were displayed continuously during the task with breaks in the form of fixation crosses between each sequence (after every 20 stimuli). These were included to provide participants with the opportunity to rest prior to the next sequence of uninterrupted trials. The four blocks of sequential stimuli lasted 7:30 minutes in total. All stimuli were presented for 2 seconds and randomised with an interstimulus interval of 1-4 seconds, randomly jittered in 0.5 second increments between stimuli. Stimuli were not repeated.

Participants were instructed that pictures of people's faces would be displayed on the screen one at a time and that they had to think about what the person is feeling as they looked at each picture. Each time a new face was presented on the screen, the participant needed to decide whether the person whose face was currently displayed on the screen felt the same way or differently from the person who was displayed on the screen in the previous picture. The instructions indicated that the aim of the task was not to (a) judge whether the person looked the same or different to the one before; (b) name the emotion/affect portrayed; or (c) determine whether the same person's face was displayed consecutively. Instead, the instructions emphasized that the goal was to decide whether the person currently on the screen felt the same or differently from the person immediately preceding, irrespective of face identity. The task is designed to ensure that subjects make the affective appraisal without attaching a verbal label to the expressed emotion. I took great care not to label or use any emotion-describing words during the instructions.

A paper practice trial was run to test the child's understanding of the instructions. Further instructions regarding button-presses were then added. The child was then instructed that once s/he had decided whether the person on the screen was feeling the same as or

different from the previous person, they have to press a designated key on the keyboard for "same" (indicating that the currently displayed person feels the same as the previous one) and another key for "different" (indicating that the currently displayed person feels different to the previous person). The target keys were marked with stickers to distinguish them from the other keys on the keyboard, and to minimize buttons other than the target buttons from being pressed.

A second paper practice trial was run in which the new instructions for pressing the "same" and "different" keys was incorporated, and the knowledge of the correct keys was assessed. Once the child had mastered the correct button pressing and understood that a key had to be pressed each time and only once, as well as demonstrated understanding of the task instructions, a practice computer trial was run simulating the actual affective appraisal computer task. Upon successful completion of the practice trial, the actual task was administered over two consecutive trials.

The presentation of this task places demands on WM as the affective category displayed needs to be temporarily maintained in memory. To prevent the potential confounding effect of introducing a more complex level of WM integration, the affective appraisal task was not administered only as a 1-back test.

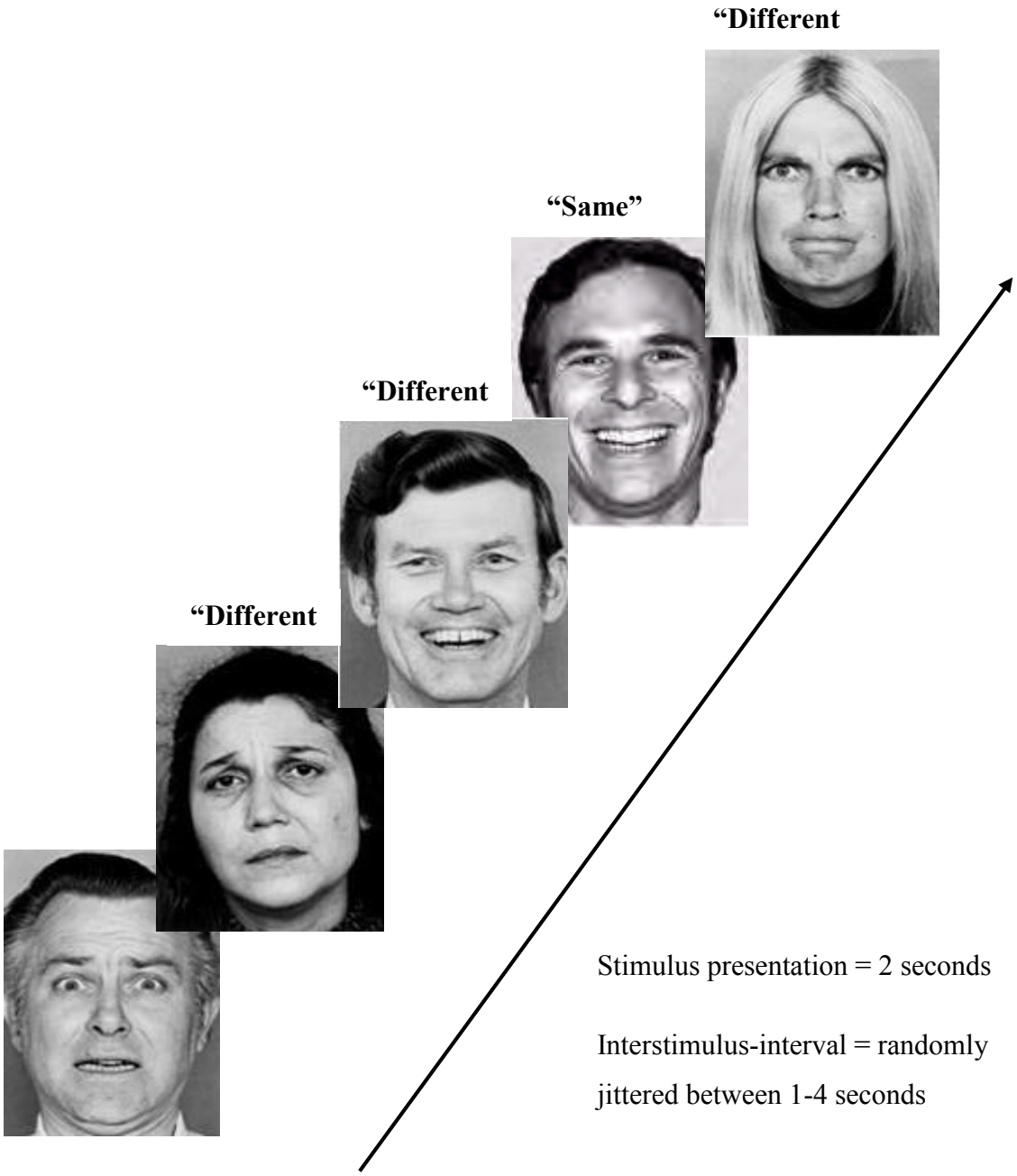

Figure 5.1. Schematic depiction of the affective appraisal task. Details of the task are outlined in the text. Response to each stimulus (face) was assessed across the diagnostic groups, contrasting alcohol exposed individuals to healthy controls. The appropriate response to each stimulus ("same"/ "different") is presented here for exposition purposes only.

Statistical Analysis

The information on alcohol and drug use collected during the prenatal interviews was used to generate continuous alcohol and drug use (marijuana, cocaine and methamphetamine) measures. To reduce the effects of outliers, these measures underwent log(x+1) transformation if they were skewed (skew>3.0). The main behavioural outcome variable generated by the WM and affective appraisal tasks is *d*-prime, which measures a participant's correct number of button presses, adjusting for false alarm presses. The number of correct button presses (hit rate) and false alarm presses (false alarm rate) were also recorded and examined separately. Between-group differences, based on FASD diagnosis, were initially examined for the WM and affective appraisal outcome measures using one-way analysis of variance (ANOVA) and then also using analysis of covariance (ANCOVA) to adjust for potential confounders, namely child age at testing, child sex, maternal education and maternal smoking during pregnancy. The effects of IQ and EF as potential mediators of the effect of alcohol were examined by including them as covariates in ANCOVAs. Correlations were run to examine the relation between AA/day (a continuous alcohol measure) and the outcome measures, independent of FASD diagnosis. For outcome measures showing a significant alcohol-related effect, additional multiple regressions were run to control for potential confounders and to investigate whether PAE effects were mediated by IQ or EF.

Results

Sample Characteristics

Table 5.1 summarises the demographic and background characteristics. During pregnancy, mothers of children with FAS and HE children drank on average 8-9 standard drinks on 1-2 days per week. Mothers of children with PFAS drank on average 8 standard

drinks 2 days per week. All of the mothers of control children abstained from consuming alcohol during their pregnancy. There was a significant between-group difference (between mothers of controls and mothers of children with PAE) on all three variables measuring prenatal alcohol consumption ($p<.001$). However, the alcohol exposed groups did not differ significantly from one another on any of the prenatal alcohol consumption variables (all ps >.280). Many of the mothers reported smoking cigarettes during pregnancy and 10 (FAS=1; PFAS=1; HE=7; controls=1) mothers reported smoking marijuana. None of the mothers reported using cocaine during their pregnancy. Smoking was investigated as a potential confounder. On average, children with FAS and PFAS were somewhat older than HE and control children ($p<.001$). As expected, WISC-IQ scores differed significantly between the diagnostic groups; children with FAS and PFAS scored significantly lower than both HE and control children.

Performances on the various EF tasks are summarized in Table 5.2. Significant between-group differences were found on 6 of the 9 EF measures. Except for the *Stroop Set Shifting* task, the FAS and PFAS groups performed similarly and more poorly than both the HE and control groups, who performed similarly. Post-hoc pairwise comparisons using the Fisher least significant differences (LSD) test detected no significant differences between the FAS and PFAS groups, or between the HE and control groups on any of EF measures. See Appendix K for the complete post-hoc analysis of all the EF tasks. Where significant differences were shown on outcome measures, either across diagnostic groups or on a measure of continuous alcohol, IQ and EF were controlled for as potential mediators of the effects of alcohol on the outcome.

Table 5.1

Sample Characteristics

	FAS (n=12)	PFAS (n =11)	HE (n =27)	Controls (n =38)	F or χ^2	p
Maternal age at delivery	29.5 (6.7)	28.9 (8.2)	24.8 (4.6)	26.0 (6.1)	2.31	0.082
Maternal education (years)	9.1 (2.0)	7.5 (3.0)	9.4 (2.4)	10.0 (2.0)	3.55	0.018
Alcohol exposure[a]						
AA/day (oz)	0.9 (0.8)	1.2 (0.8)	1.2 (1.2)	0.0 (0.0)	12.54	<0.001
AA/occasion (oz)	4.4 (2.2)	4.0 (1.3)	4.3 (3.0)	0.0 (0.0)	26.88	<0.001
Frequency (days/week)	1.4 (0.7)	2.1 (1.4)	1.4 (1.4)	0.0 (0.0)	25.68	<0.001
Prenatal smoking	5.8 (3.2)	8.9 (6.5)	8.1 (5.8)	5.5 (4.6)	1.41	0.251
Child						
Age at testing (years)	12.0 (1.4)	12.7 (1.5)	10.5 (0.5)	11.0 (1.1)	13.6	<0.001
Sex (% male)	50.0	63.6	40.7	42.1	1.98	0.576
WISC-IV IQ[c]	63.6 (8.9)	64.2 (10.2)	75.8 (16.0)	76.8 (14.6)	4.53	0.005

Values are mean (SD) or %.

[a]Consumers only (FAS: n = 12; PFAS: n =11; HE: n = 22).

[b]Smokers only (FAS: n = 10; PFAS: n =11; HE: n = 23; Controls: n = 17).

[c]Wechsler Intelligence Scale for Children-Fourth edition.

Table 5.2

Executive function scores across the four diagnostic groups

Measure	FAS	PFAS	HE	Controls	*F*	*p*
Working Memory						
WISC Digit Span Backwards	4.7 (1.6)	4.7 (2.2)	6.0 (2.2)	6.0 (1.5)	2.88	0.041
Goal Setting						
Tower of London	2.6 (0.8)	2.5 (1.4)	2.9 (1.6)	2.7 (1.1)	0.42	0.738
Attentional Control						
Rubia Stop	331.8 (133.0)	289.6 (105.9)	222.3 (81.2)	219.3 (97.5)	4.15	0.009
Stroop Set Shifting (sec)	123.7 (34.0)	103.9 (6.4)	94.4 (22.2)	102.6 (21.7)	3.99	0.011
Cognitive Flexibility						
Stroop Interference (sec)	90.8 (15.9)	98.5 (24.9)	90.4 (21.0)	89.7 (15.5)	0.56	0.645
Children's Colours Trail Task	1.0 (0.6)	1.0 (1.2)	1.0 (0.6)	0.9 (0.6)	0.10	0.962
Verbal Fluency						
Verbal Fluency Letters	13.6 (5.1)	11.7 (6.2)	20.5 (8.9)	18.5 (9.3)	3.38	0.022
Verbal Fluency Categories	20.8 (6.6)	18.4 (4.8)	26.2 (7.9)	26.4 (5.8)	5.61	0.002
Verbal Fluency Switch	8.1 (1.7)	8.6 (2.8)	10.9 (2.2)	10.8 (2.0)	6.78	<0.001

Note. Values are mean (SD).
The number of participants completing each EF task varied slightly across groups for each task. See Appendix K for further details on group sizes.
Significant group differences were seen for: Rubia Stop (FAS > HE; FAS > Controls); Digit Span (FAS < HE; FAS < Controls; PFAS < HE; PFAS < Controls); Verbal Fluency Letters (FAS < HE; PFAS < HE; PFAS < Contorls); Verbal Fluency Categories (FAS < HE; FAS < Controls; PFAS < HE; PFAS < Controls); Verbal Fluency Switch (FAS < HE; FAS < Controls; PFAS < HE; PFAS < Controls) and Stroop Set Shifting (FAS < HE; FAS < Controls). See Appendix K for *p*-values of the post-hoc analysis.

Working Memory Performance across the Diagnostic Groups

Of the 88 children assessed, 81 were able to complete the 1-back WM task. Of that number, 7 (FAS=2; HE=4; Controls=1) did not complete the 2-back task as they were unable to understand the more complex next step. Group differences on performance of the more complex 2-back WM task were explored using the remaining 74 participants who successfully demonstrated understanding of the task. Table 5.3 summarizes the outcome measures for the 1-back and the 2-back task.

Although there were no between-group differences on the 1-back WM task, $F(3,77) = 0.26$, $p>.20$, $\eta 2=0.010$ (see Figure 5.2) FASD diagnosis was associated with poorer performance on the 2-back task, $F(3,70) = 2.98$, $p=.037$, $\eta 2=0.113$. Post-hoc pairwise comparisons using the Fisher LSD test showed that, specifically, the children with FAS performed more poorly than the HE ($p=.008$) and control ($p=.013$) groups. However, there were no significant differences between the PFAS group and any of the other groups, or between the HE and control group (all ps$>.10$).

The hit rate and false alarm presses showed no significant group differences on either the 1-back or the 2-back. Response bias was measured between the groups in order to examine a potential difference in responding biased either towards "same letter" or biased towards "different letter". The response bias measure was comprised of the average measure of the bias criterion metric c (Macmillan & Creelman, 2005). No between-group differences were observed regarding biased responses on the 1-back ($F(3,77) =1.93$, $p>.10$, $\eta 2=0.070$) and on the 2-back ($F(3,70) =0.47$, $p>.10$, $\eta 2=0.20$). However, groups displayed a slight bias to respond with "different letter", which is to be expected given that the majority of correct responses is "different letter".

Table 5.3

Behavioural Outcome Scores for Working Memory and Affective Appraisal

Measure	FAS	PFAS	HE	Controls	*F*	*p*
1-back (*n*=81)[a]						
D-prime	2.73 (0.74)	2.64 (0.69)	2.55 (1.18)	2.46 (0.87)	0.26	0.854
Hit rate	0.74 (0.18)	0.80 (0.13)	0.76 (0.19)	0.75 (0.18)	0.51	0.678
False –Alarm rate	0.03 (0.02)	0.05 (0.04)	0.07 (0.09)	0.05 (0.05)	1.40	0.249
Response bias	0.59 (0.41)	0.39 (0.25)	0.40 (0.31)	0.55 (0.25)	1.93	0.132
2-back (*n*=74)[b]						
D-prime	0.93 (0.59)	1.28 (0.56)	1.62 (0.56)	1.55 (0.73)	2.98	0.037[*]
Hit rate	0.44 (0.17)	0.53 (0.23)	0.56 (0.16)	0.55 (0.19)	1.56	0.207
False –Alarm rate	0.18 (0.17)	0.13 (0.08)	0.10 (0.07)	0.12 (0.09)	1.56	0.207
Response bias	0.64 (0.51)	0.56 (0.43)	0.62 (0.39)	0.51 (0.38)	0.47	0.701
Affective Appraisal (*n*=84)[c]						
D-prime	0.60 (0.66)	0.61 (0.71)	0.69 (0.53)	0.71 (0.55)	0.16	0.920
Hit rate	0.35 (0.21)	0.49 (0.26)	0.45 (1.89)	0.45 (0.16)	1.13	0.340
False –Alarm rate	0.15 (0.06)	0.30 (0.21)	0.22 (0.13)	0.22 (0.13)	2.45	0.070
Response bias	0.79 (0.43)	0.37 (0.77)	0.53 (0.51)	0.50 (0.37)	1.46	0.231

Values are mean (SD).

[a]FAS=11; PFAS=10; HE=25; Controls=35.

[b]FAS=9; PFAS=10; HE=23; Controls=32.

[c]FAS=11;PFAS=11; HE=26; Controls=36.

[*]Significant difference on 2-back d-prime where FAS < HE and FAS < Controls.

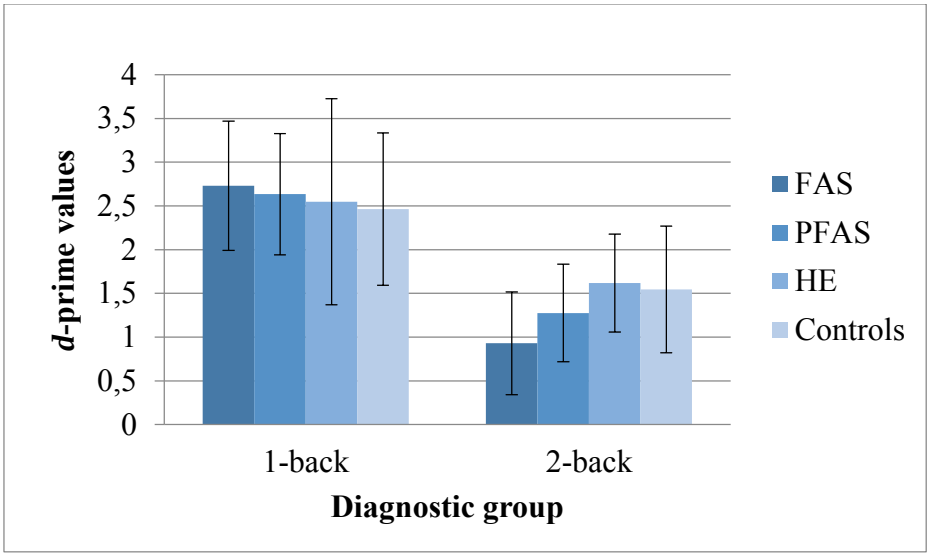

Figure 5.2. Performance on *n*-back working memory task by diagnostic group. 1-back (FAS=11; PFAS=10; HE=25; Controls= 35); 2-back (FAS=9; PFAS=10; HE=23; Controls= 32); error bars show 1 standard deviation above and below the mean

Investigation of potential confounders and mediators. The only significant between-group differences were found on the 2-back WM task. Hence, we examined whether there were any potential confounders or mediators that impact the effect of alcohol on that task. A correlation analysis showed that none of the potential socio-demographic confounders listed above met the criterion for inclusion in the analyses (all *p*s > .10; see Appendix L for correlation matrix). Hence, none were further considered as confounders of the effect of alcohol on the 2-back WM task.

Performance on tasks measuring IQ and various EF domains (WM, goal setting, attentional control, cognitive flexibility and verbal fluency) was subsequently examined as a potential mediator of the effects of alcohol on the outcomes. Scores for IQ and each EF domain were added as covariates during separate ANCOVAs. Whereas previously there were significant between-group differences on the *d*-prime outcome measure of the 2-back WM task, the ANCOVA showed that once EF was controlled for this effect was no longer

significant: working memory ($F(3,69) = 1.64$, $p=.187$), goal setting ($F(3,66) = 2.41$, $p=.075$), attentional control ($F(3,68) = 2.13$, $p=.105$), cognitive flexibility ($F(3,66) = 2.54$, $p=.064$) and verbal fluency ($F(3,67) = 1.21$, $p=.312$). Furthermore, the alcohol effect seen on the 2-back WM task was also mediated by IQ ($F(3,69) = 1.23$, $p=.305$).

WM, as measured by the 2-back task, is an EF ability essential to many cognitive processes. Hence, it was not surprising that performance on this task was mediated by all of the EF domains measured. As to be expected, performance on the Digit Span Backwards task, here used to measure working memory as an EF, was moderately correlated with the *d*-prime outcome on both the 1-back ($r=.31$, $p<.01$) and 2-back tasks ($r=.34$, $p<.01$).

Affective Appraisal Performance across the Diagnostic Groups

Four (FAS=1; HE=1; Controls=2) of the 88 children assessed were unable to complete the affective appraisal task. Differences in performance were, thus, examined using the 84 participants who successfully completed the task.

As shown in Table 5.3, the analysis detected no significant between-group differences on the main outcome measure, *d*-prime, of the affective appraisal task, $F(3,80) = 0.16$, $p>.20$, $\eta^2=0.006$ (see also Figure 5.3). However, there was a trend toward significance on the False-Alarm rate outcome measure. Post-hoc pairwise comparisons using the Fisher LSD test showed that the PFAS group exhibited a significantly higher false alarm rate than the FAS ($p=.008$) group, but not than the HE ($p=.098$) and control groups ($p=.074$; Figure 5.4), suggesting greater impulsivity. By contrast, the FAS group had a significantly higher rate of correct rejections compared to the PFAS group ($p=.007$) but not to the HE ($p=.086$) and control groups ($p=.058$; Figure 5.5), possibly due to their slower response rates. To investigate potential differences in responding biased either towards "same" or biased towards "different", response bias was compared between the groups. No between-group differences were observed regarding biased

responses ($F(3,80) = 1.46$, p=.231, η^2=0.052), with all groups, however, displaying a slight bias towards responding "different". As indicated earlier, this is to be expected given that the majority of correct responses were in fact "different".

Responses on the affective appraisal task were also investigated for differences related to the valence (e.g., happy, sad, angry, etc) of the affect displayed in a face. The only emotion on which the groups differed consistently was the appraisal of angry faces. The PFAS group was less likely to correctly identify an angry face than the FAS, HE, and control groups. When angry affect was displayed, children with PFAS had more false alarms (i.e., failure to recognise an angry face as angry) compared to the FAS (p=.010) and HE (p=.039) groups. Furthermore, for angry faces, the PFAS group had significantly fewer correct rejections (i.e., recognizing angry affect as angry) compared to the FAS (p=.004), HE (p=.023), and control (p=.031) groups.

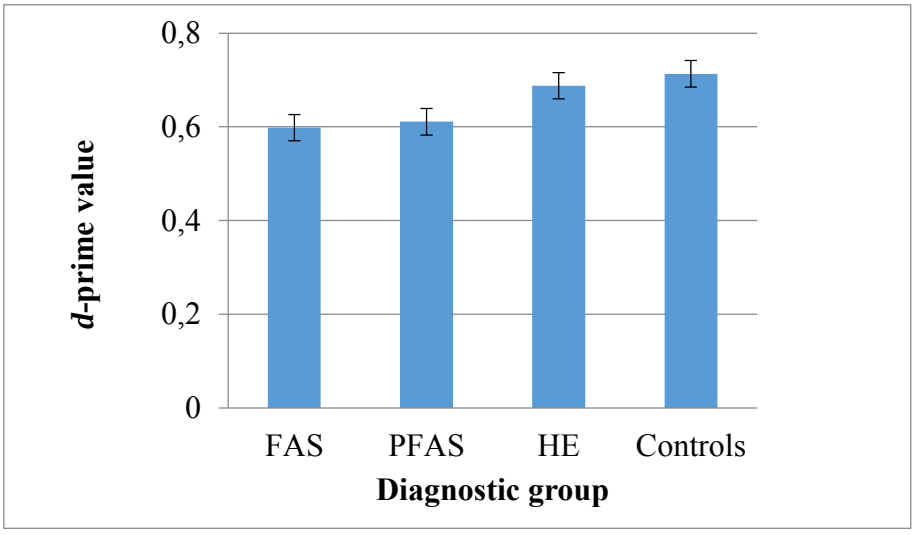

Figure 5.3. Affective appraisal performance by diagnostic group.
FAS=11; PFAS=11; HE=26; Controls= 36.

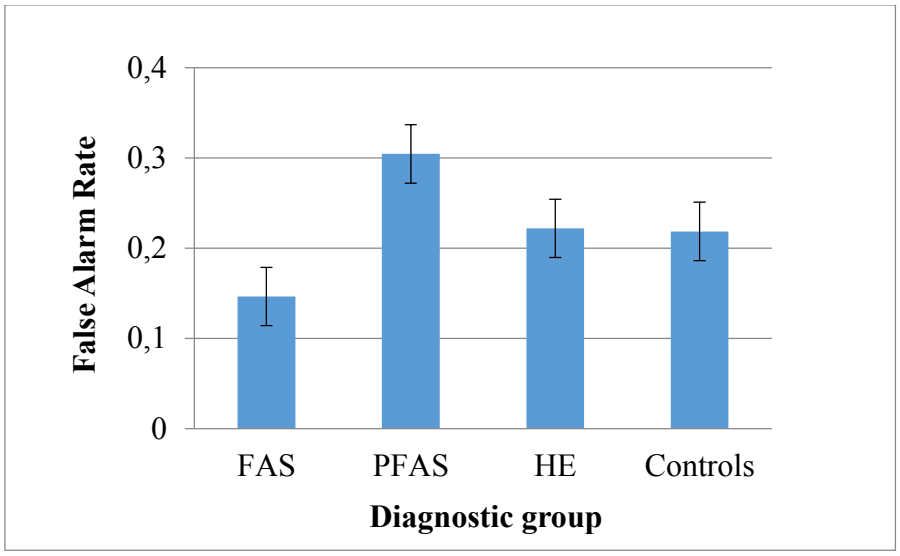

Figure 5.4. Affective appraisal false-alarm rate by diagnostic group.
FAS=11; PFAS=11; HE=26; Controls= 36.

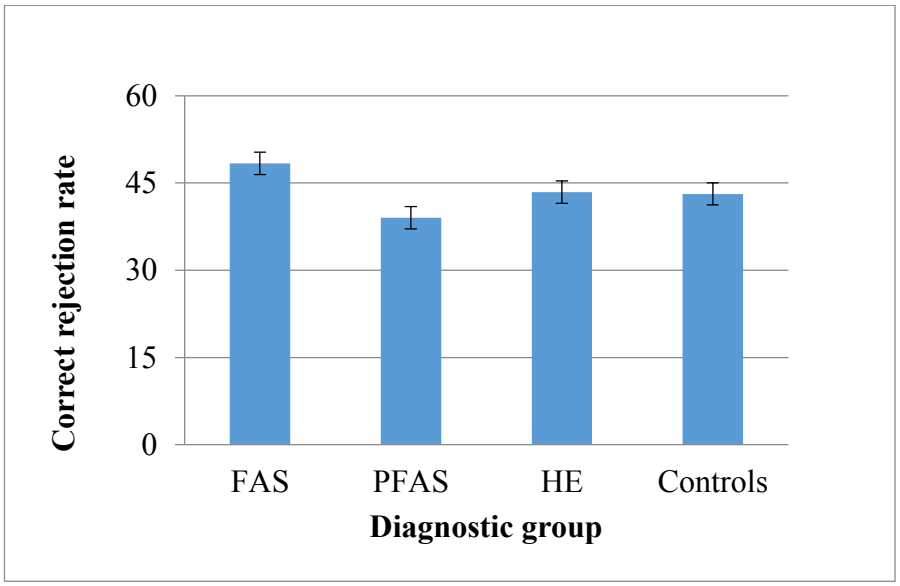

Figure 5.5. Affective appraisal correct rejection rate by diagnostic group
FAS=11; PFAS=11; HE=26; Controls= 36.

Relation of Continuous Alcohol to Outcome Measures

AA/day was not related to the *d*-prime outcome measure on the 1-back (r=.02; p=.866), 2-back (r=-.01; p=.951), or affective appraisal (r=.03; p=.821) task and was thus not further investigated.

Working memory impact on affective appraisal

The affective appraisal task follows a 1-back format. There were no between-group differences on the 1-back WM task (see Table 5.3), indicating that all four groups demonstrated the ability to meet the WM demands of the affective processing task. There was a moderate correlation between the performance on WM 1-back and 2-back tasks (r=.50, p<.001), but neither was related to affective appraisal (rs=.06-.08), indicating that WM *per se* did not influence performance on the affective appraisal task. As the investigations of Study II were primarily motivated by the findings of the RME task in Study I of the same cohort, performance on the affective appraisal task was correlated with that of the RME test. Performance on affective appraisal was moderately related to performance on the RME test (r=.47, p<.001), indicating measurement of a common domain. (See Appendix M for a scatterplot of the relation between the two tasks).

Discussion

The main focus of this study was to further investigate potentially impaired aspects of social cognition in FASD, following results obtained in Study I. The results of that study were consistent with the idea that PAE is associated with impairment in the ability to appraise the emotional/mental state of others from facial/eye expressions. In Study II, the primary aim was to establish whether there are significant between-group differences in children with FASD on an affective appraisal task, which may be indicative of difficulties in reading social cues from

faces. As a secondary aim, I also assessed WM ability in order to examine its effect on affective appraisal.

Most importantly, the results of Study II were able to successfully answer the major research questions I wanted to address in this design. In this regard, all diagnostic groups were able to successfully complete a 1-back WM task. Consistent with previous research (Diwadkar et al., 2013), as complexity of the WM demands increased from 1-back to 2-back, children with FAS performed significantly more poorly than the other groups. These data confirm the hypothesis that children with FASD perform more poorly on the WM task compared to healthy controls, particularly at higher levels of cognitive complexity. Also, these data confirm that children with PAE are able to complete less complex WM tasks successfully but that as complexity increases, impairments become more evident. This pattern of data suggests that cognitive tasks that place minimal demands on WM capacity may be less affected by the effects of PAE.

Although there was a moderate association between the 1- and 2-back WM tasks, neither were related to affective appraisal, indicating that WM capacity did not influence performance on the affective appraisal task. Performance on the 1-back WM task confirmed the participants' ability to retain the information needed to perform the affective appraisal task, because this task also uses 1-back design. Moreover, a correlation analysis suggested that the RME task and the affective appraisal task appear to measure the same functional domain.

Performance on affective appraisal itself was the primary focus of this study. Overall, analyses detected no effect of PAE on the affective appraisal task performance, using either diagnostic group comparisons or a measure of continuous alcohol. These findings are consistent with the results of two previous studies that used this task. Neither found

performance differences between healthy controls and participants at risk for schizophrenia aged 8-20 years (Barbour et al., 2010; Diwadkar et al., 2012).

However, when considering the valence of the emotions expressed in the affective stimuli, differences emerged between the FAS and PFAS groups. These differences demonstrated here are consistent with the notion that the PFAS group responded more impulsively and less accurately, especially when their performance on trials featuring angry stimuli are contrasted to those featuring non-angry stimuli. These findings are, furthermore, significant as they demonstrate that PAE shows different patterns of association depending on the valence of the emotion, and may thus differentially impact on facial affective appraisal.

This valence-specific finding is consistent with research showing emotion recognition deficits in other atypically developing children. For instance, compared to typically developing individuals 8-to-12-year-old boys with ADHD have difficulties in accurately identifying angry facial expressions (Ichikawa et al., 2014). The same study demonstrated that, this effect for angry emotions stood in contrast to findings on the identification of happy facial expressions, where participants with ADHD performed similarly to typically developing controls. Similarly, consistent with the performance of typically developing preschool children, young school-aged children with Down syndrome (mean chronological age: 76.7 months) have greater difficulties in labelling facial emotions being displayed than when matching a displayed emotion to its label. Both typically developing and children with Down syndrome appear to have difficulties in both labeling and recognizing facial emotions of anger and fear, but found the processing of happy and sad facial expressions easier (Kasari, Freeman, & Hughes, 2001).

Limitations

The affective appraisal task used here is a relatively simple means of assessing the way in which individuals process affective facial signals. Thus, it was perhaps not surprising that the analyses detected no overall between group differences. However, one may speculate that this lack of difference may be related to the age range of the participants, and that differences in performance may be present when assessing younger children. Also, based on the findings on the 2-back WM task, one may expect that if the affective appraisal task's complexity were increased (e.g., to follow a 2-back design) between-group differences may indeed emerge. Thus, future investigations of affective appraisal may want to consider implementing a more complex task or assessing this ability in younger children with FASD, or both.

A possible further limitation of the affective appraisal task relates to the affective stimuli used. All affective faces were taken from a database (Ekman & Oster, 1979) that features using only White individuals, whereas all participants of the current study were of mixed-ancestry (Cape Coloured) decent. This cross-race judgment of emotions becomes problematic considering evidence suggesting that emotions may be more easily recognized when they are evaluated within the same race or regional group (Elfenbein & Ambidi, 2002). However, a meta-analytic review suggests this in-group advantage is smallest for the recognition of happy and angry emotions (Elfenbein & Ambidi, 2002). Although acknowledging the cross-race biased task stimuli, this task was selected nonetheless because no validated local, South African, stimuli are currently available. Hence, although the task required cross-race affective appraisal, comparisons were made between participant groups, who were all recruited from the same ethnic group and community. The fact that all participants share the same demographic environment may, thus, control for a potential cross-race effect. Ideally, however, future studies

should use a task featuring same-race stimuli validated and suitable for the multi-cultural South African setting.

Directions for Future Research

Study II was designed to further investigate the between-group differences, observed in the Study I RME data, in the ability to read other's mental states. Findings on the affective appraisal task data detected no significant between-group differences on the main outcome measure, however. One potential explanation for these negative finding is that neuroimaging research has shown that a similar behavioural outcome can be related to different underlying neural processes in different children, particularly those whose brain function is impaired. For example, children with PAE appear to recruit a broader range of neuroanatomical regions (Meintjes et al., 2010) or different neuronal networks (Diwadkar et al., 2013) when performing the same task non-exposed peers. This literature suggests that children with PAE employ different compensatory tactics to achieve the same behavioural or cognitive outcome. Identification of the specific underlying neural processes seen in children with PAE can provide the basis for designing behavioural and cognitive intervention paradigms that are specifically targeted at the appropriate neuronal endpoints.

Even though analyses detected no between-group differences on the affective appraisal task, results from Study I suggested that children with FASD do indeed have deficits in the facial emotion recognition domain. Given these findings and what we know about neuroimaging in FASD populations, investigating potential differences in the recruitment of neuroanatomical structures during the process of affective appraisal may provide insight into a possible relation of PAE and the ability to read affective facial cues. Future research could, thus, be aimed at establishing whether during the processing of affective cues, children with

FASD recruit a different, or a more extensive, cortical network to achieve the same outcome as their non-exposed peers.

Introducing the rationale for Study III. The findings reviewed above provide the premise for Study III, in which I investigate cortico-limbic activation during affective appraisal in a neuroimaging protocol using a functional magnetic resonance imaging (fMRI) adapted version of the same affective appraisal task as used in Study II. Below I explain the rationale that helped to establish this research plan. The RME task used in Study I required that the child choose one of four possible responses. Thus, if the RME task were to be used, a participant would be required to press one of four buttons while in the magnetic resonance imaging (MRI) scanner, one corresponding to each possible response. The consideration of the following factors led me to conclude that using the RME task in the scanner would introduce too much error: (a) the complexity of learning and remembering the location of four buttons to be pressed during the scan, when the response box is not visible; (b) complexity of using a four-button response paradigm for these children; (c) potential between-group differences in reading ability; and (d) inability to ask for definitions of the stimuli words/phrases, an integral part of the behavioural assessment, while in the scanner. None of these factors were of concern during the behavioural administration of the RME task in Study I, as there were no button presses and stimuli words were read by the examiner, and where necessary, explained to the participant. Furthermore, the large between-group differences on the behavioural performance of the RME would make it difficult to interpret differences in fMRI results as groups are likely to differ behaviourally. The RME task in its current format was, thus, deemed unsuitable for use in an fMRI protocol. Instead, the affective appraisal task (which appears to measure the same social-cognitive domain as the RME), was considered to be suitable for examining effects of PAE during fMRI, for the reasons outlined below.

Equating performance between groups is critical for the interpretation of functional neuroimaging data. If performance between groups is significantly different, it is difficult to know whether a group difference in neural activity was a cause, or simply a consequence of, the difference in performance. Analyses detected no between-group differences in performance on the affective appraisal task overall. Since behaviourally during Study II, all four participant groups were able to perform the task successfully, results from the scanner would most likely be the same. Hence, the neuroimaging data component could then be analysed and any significant findings interpreted as being indicative of the underlying mechanisms related to the affective response during the actual neural process. In support of this notion are previous neuroimaging studies (Barbour, et al., 2010; Diwadkar et al, 2012) that used the same affective appraisal task to investigate cortico-limbic activation in individuals at risk for schizophrenia to healthy controls. Both these studies did indeed find between-group differences in cortical activations and network recruitment, even though behaviourally participants did not differ in task performance.

In addition, the valence-related effect observed in this study suggests that there should be further investigation of potential differences in the recruitment of neuroanatomical structures and networks that are specific to the valence of the affective stimuli and, thus, warrants further investigation. Therefore, a neuroimaging study could also establish whether affective processing in individuals with FASD differs depending on the valence of the affect that needs to be processed. In support of such an investigation are findings by Ichikawa and colleagues (2014) who found by looking at hemodynamic responses that children with ADHD show atypical brain activity for the processing of angry faces but not for that of happy faces.

Hence, Study III was designed to identify and characterize potential compensatory mechanisms or atypical processing styles during affective appraisal in children with FASD.

The following chapter presents the findings of Study III, which employed behavioural and brain imaging paradigms to investigate potential differences in cortico-limbic activations. Within that chapter the rationale for this final study is further discussed within an overview of the neuroimaging technique and data processing methods applied, concluding with an interpretation of the final results.

CHAPTER SIX: Study III - Prenatal alcohol exposure and affective appraisal: Patterns of cortico-limbic activation in children with fetal alcohol spectrum disorders

Introduction

Our facial expressions provide the people around us with social cues portraying information about our emotional state, which in turn provides social signals regarding, for example, our approachability or intent. 'Reading' another's face facilitates the interpretation and understanding of a social situation and allows others to react in what is deemed to be a socially appropriate manner. Hence, the affect displayed in a face is a major factor that mediates our social interactions. However, appropriate social behaviour depends on correctly interpreting a person's facial expression and subsequently reacting accordingly. Prior to the interpretation of an affective stimulus, the stimulus needs to be correctly identified. The ability to successfully and accurately perceive an emotion portrayed by a face is highly dependent on intact functioning of numerous underlying cortical processes. Face perception involves an array of complex neuronal processes, which include establishing facial identity and familiarity and the recognition of facial expressions and emotions (Adolphs, 2002).

Detrimental effects of PAE on the developing brain and the resulting structural and functional abnormalities have been well documented (see *Chapter One*, pp. 5-14). Studies have shown that individuals with FASD also have global and localised anatomical brain differences compared to non-exposed individuals. Information regarding these anatomical differences and resulting differences in neural activation patterns may offer important insight into the cognitive and behavioural impairments associated with FASD.

In the previous chapters, I presented findings on social-cognitive ability in children with FASD, in particular their performance on ToM and affective appraisal. In addition to evaluating performance on affective appraisal comparing children with FASD to controls, Study II suggested suitability of the affective appraisal task for use in a MRI scanner. In this chapter, I present the findings from Study III of my dissertation, a neuroimaging study investigating affective appraisal in children with FASD using fMRI. This chapter (1) provides a brief overview of the neuroimaging technique and methodology employed in this study; (2) reviews the neurocognitive profile of FASD based on previous findings from fMRI research, before presenting the general research aims of the current study; (3) detail the data acquisition and analysis and presents the overall findings and a discussion of the results.

Introduction to Functional Magnetic Resonance Imaging

The neuroimaging technique most frequently used to study regional brain activation is fMRI. A non-invasive technique, it uses the measurement of the blood oxygenation levels during neuronal activation to characterise the functional involvement of specific neuroanatomical regions and networks. In short, fMRI involves acquiring images based on the measurement of the brain's hemodynamic response, i.e., the increase in blood flow to an activated area (Buxton, Uludag, Dubowitz, & Liu, 2012). Using the brain images that were acquired, one can examine the changes in brain function over time. Following the image acquisition is the interpretation of the difference in the magnetic susceptibility of the hemodynamic response to a specific stimulus, or sequence of stimuli, at a specific time. This is known as the hemodynamic response function (HRF; Lindquist, 2014). A graphical representation of the HRF is shown in Figure 6.1. This technique involves comparing the hemodynamic response in reaction to a stimulus presentation to that seen during a baseline condition. The baseline condition needs to be designed in such a way that it elicits the same

attentional, perceptual, and motor processing as the stimulus condition, but differs in terms of the cortical processing studied.

This blood oxygenation level dependent (BOLD) imaging technique employed during MRI collects information related to the changes in the oxygenation levels of the haemoglobin present in brain regions when a region in the brain is activated, as is the case when an individual engages in a task in a MRI scanner. Depending on the concentration of the oxyhaemoglobin (a higher concentration gives a higher signal, which in turn generates a brighter image; vice-versa for a lower concentration), the BOLD images acquired will document the changes in brightness of certain areas in the brain, i.e., show the different levels of oxyhaemoglobin (Lindquist, 2014). Regardless of the metabolic demands of breaking down the oxygen required in the activated brain region, the neuronal function is related to an increase in oxyhaemoglobin concentration due to an increase in blood flow. This in turn, leads to a decrease of the dexoyahemoglobin concentration. Because more oxygen is supplied than is consumed, an increase in the ratio between brain tissue containing deoxygenated haemoglobin (due to ongoing metabolic processes) and brain tissue containing oxygenated haemoglobin (due to an increase in blood flow triggered) causes a high BOLD signal (i.e., produces a brighter image).

The onset of this higher signal occurs about 2 seconds after neural activation, due to the time it takes for blood to flow into and out of the region. The signal peaks about 5-8 seconds after the peak of the neuronal activation (Aguirre, Zarahn, & D'Esposito, 1998). Following the peak, the BOLD signal drops to below baseline, which is known as the post-stimulus undershoot. This occurs because following the initial neural response to the stimuli, blood flow to that region decreases more quickly than blood volume, which results in a

greater concentration of deoxyhaemoglobin. If there are no additional stimuli causing neural activation, the BOLD signal returns to baseline about 20 seconds later.

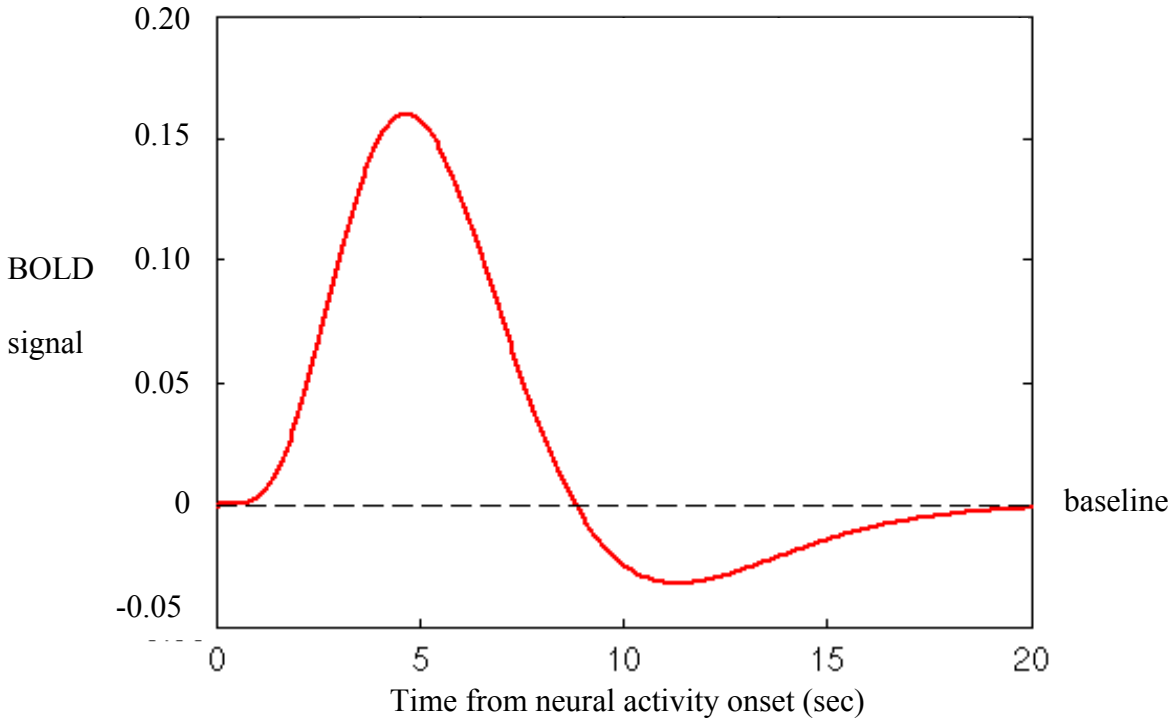

Figure 6.1. Haemodynamic response function

The different magnetic properties of oxyhaemoglobin and deoxyhaemoglobin make it possible to measure the BOLD signal, allowing the generation of a neuronal activation map, showing which brain regions were activated during the task and which were not (Amaro & Barker, 2006).

Experiment design. In studies designed to examine neural activation during cognitive processing, to acquire the BOLD images during fMRI, a participant needs to engage with a task while lying in the MR scanner. There are various techniques that can be employed to present the stimuli to the participant, and their selection is dependent on the experimental

paradigm. The most common types of experimental designs are block design and event-related design (see Figure 6. 2; Lindquist, 2014).

In the early days of fMRI research, experiments used a simple block design (Huettel, 2012). Typically, stimuli would be presented as two different conditions and would, for example, alternate between the presentation of one block of an experimental condition for a set period, with a block of a control task being displayed for a similar amount of time (Lindquist, 2014). This method allows researchers to examine the difference between activation patterns during the experimental and control conditions and is based on averaging the brain's response across each block. The level of activation, i.e., the relative change in signal intensity, in each block or condition is then compared between the tasks or between an experimental condition and the resting condition; i.e., when no stimulus is displayed (Amaro & Barker, 2006).

An alternative design, and the experimental approach that revolutionised fMRI research, is known as event-related design. Rather than a block design, an event-related experiment allows for the detection of changes in the BOLD hemodynamic response to specific points (i.e., events) in time, and the stimuli presentation is randomised, rather than presented in a set sequence (Huettel, 2012). This approach allows for the observation of neural activity to a very specific event as the hemodynamic response is assessed following each stimulus i.e., allowing for the temporal characterisation of the BOLD signal changes (Buxton et al., 2004). Each trial of an event-related experiment may consist of different events, such as the presentation of a stimulus (for a certain amount of time), the participant's response (e.g. a button press) and a delayed period between stimuli presentations. The benefit of an event-related design is that this method is a more sophisticated technique, which allows for trial-based experiments during which both the presentation order (Rosen, Buckner & Dale,

1998) of stimuli and the interval between stimuli (D'Esposito, Zarahn & Aguirre, 1999) can be randomized. However, analysis of the data acquired by means of an event-related design, as with any fMRI data, is complex.

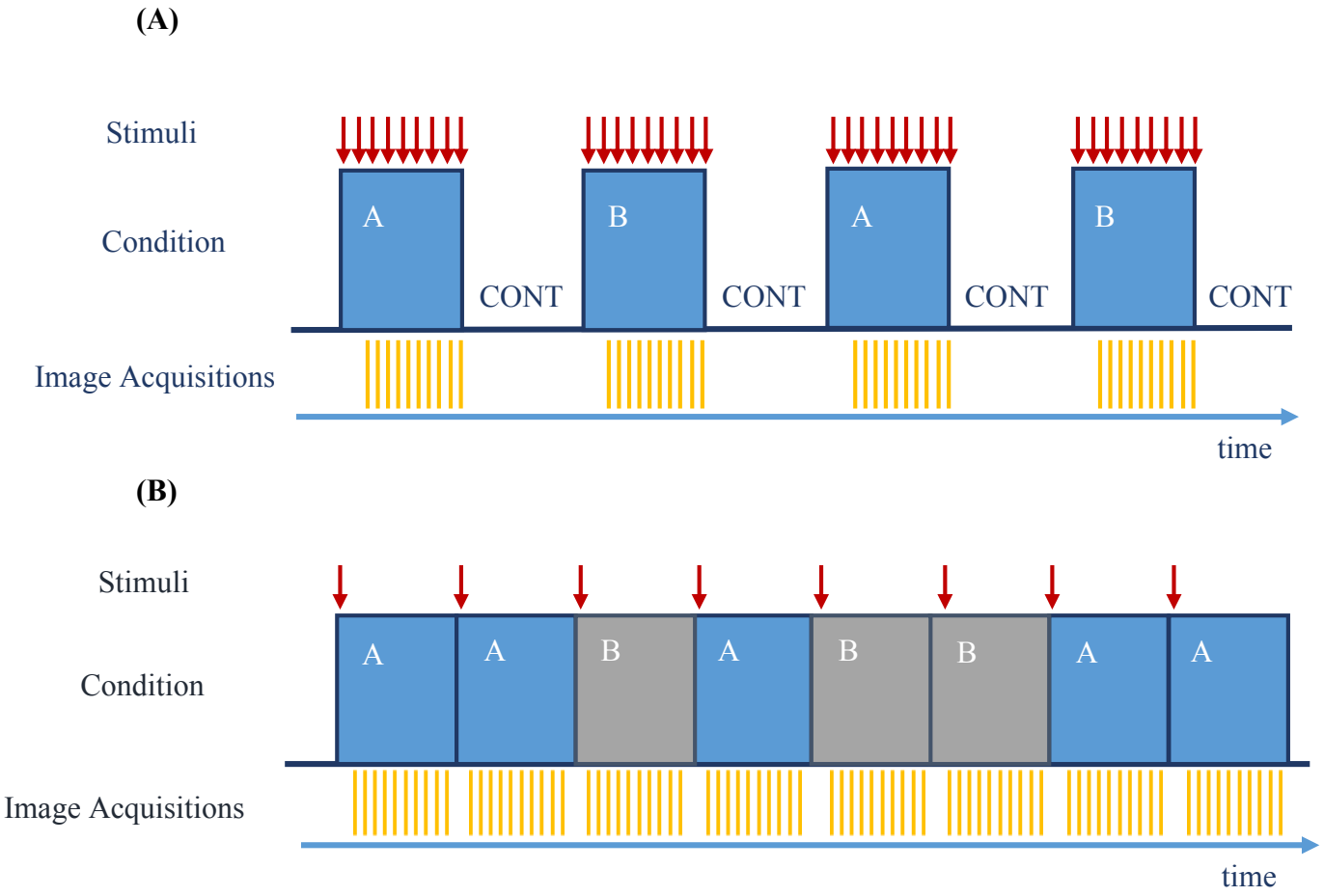

Figure 6.2. (A) Classic block design where experimental conditions (A&B) and control condition (CONT) are alternated; (B) event-related design where experimental condition (A&B) are presented sequentially within a trial.

Data Acquisition

Designing an fMRI experiment is challenging, and many variables need to be considered. One major consideration is finding a balance between adequate temporal and spatial resolution, which will be influenced by the research question (Lindquist, 2014). Temporal resolution relates to the timing of the image acquisition, i.e., the time taken to produce a brain slice image. It is regulated by a parameter referred to as repetition time (TR), which indicates the time taken to acquire (i.e., record the image of) one brain volume, which is composed of many slice images. The shorter the TR, the fewer the number of slices collected and, therefore, less information is collected. Spatial resolution is measured in voxels, which is a unit of the amount of brain tissue recorded in each image. Increasing the spatial resolution (i.e., decreasing the voxel size) will increase the TR (Amaro & Barker, 2006). The TR and voxel size are determined dependent on the experimental aims, but general recommendations for fMRI are a spatial resolution of 3 x 3 x 5 mm^3, with an image dimension of 64 x 64 x 30, which can be acquired in about 2 seconds. Because oxygenation patterns change around 5-8 seconds after activation, a TR of 2 seconds is generally accepted as suitable (Amaro & Barker, 2006; Lindquist, 2014).

Data Analysis

After completion of the data acquisition, the image scans need to pre-processed prior to conducting statistical analyses. The following pre-processing steps are generally performed: (a) slice timing correction, (b) realignment/motion correction, (c) coregistration of the structural and functional scans, (d) normalisation, and (e) smoothing. Slice timing correction relates to the fact that a brain volume consists of a series of scans on individual slices, which are not measured simultaneously. In this pre-processing step, each voxel is shifted to correct for the difference in the time of acquisition. During motion correction, the

amount of head movement that occurred during acquisition of the image is factored into the image preparation. This step is achieved by adjusting each voxel's signal, so that one can be certain that the signal received from a specific voxel is really that voxel's signal and not that of a neighbouring voxel. Motion correction is achieved by aligning the input image (each individual brain volume) with a target image (e.g., the mean structural image for the sample as a whole). The input image is then converted and rotated along anatomical coordinates and aligned with the target image. During re-alignment, new motion corrected voxels are determined using interpolation to create a resampled input mage. However, if the amount of motion during a scan is too severe for motion correction to be applied, a participant may be excluded from the analysis.

Due to the poor spatial resolution, fMRI has very poor anatomical details. Hence, the fMRI data are co-registered; that is, the acquired functional images are mapped onto each subject's detailed structural anatomical MR scan. To compare functionality across participants, each voxel needs to lie within the same anatomical structure in each participant's scans. However, due to the fact that brains vary slightly in shape, each participant's anatomical data need to be registered onto the same standardised map or brain template. This step is known as normalisation; it allows for inferences and comparisons to be made across participants or between participant groups. During smoothing the functional images are convolved using a Gaussian kernel to improve registration of neighbouring data points and to reduce the signal to noise ratio.

Following the completion of pre-processing, first-level analysis is set up using general linear modelling (Amaro & Barker, 2006) in which cortical activation within each group is examined. During this step of data analysis, activation maps or statistical parametric maps are produced aimed at identifying regions that show significant change in activation in response

to the task condition. Here, the various regressors of interest are contrasted against each other by defining a *t*-contrast for each one. In other words, looking at specific effects by contrasting different levels of the task conditions against each other. In statistical terms, at this level each pixel in the functional image is assigned a value related to the likelihood of the null hypothesis being false. The null hypothesis states that the observed signal change can be explained purely by random variation in the data consistent with its variance. Following the generation of such within-group activation maps that show significant activation in clusters surviving at the specified significance level, during second-level analysis these activation maps are then compared between the different groups. More details of the various data analysis steps and the specifications entered for processing the data of the current research study is given in the Methods sections below.

Neuroanatomical Changes Following PAE

Adverse Behavioural and cognitive deficits associated with PAE are at least partly attributable to the developing brain's vulnerability to alcohol. This vulnerability is caused by a number of mechanisms. Alcohol can cross the placenta and exert a teratogenic effect, thereby negatively impacting the normal fetal brain development. Adverse effects include cell death (Bonthius & West 1990; Ikonomidou et al., 2001), alcohol-induced hypoxia (Mitchell, Paiva, Moore, Walker, & Heaton, 1998,) or disruption of the proliferation and migration of cells (Miller, 1986).

Early research on the neuroanatomical consequences of PAE was conducted post-mortem. A range of autopsy studies found, amongst other things, cases of agenesis or malformation of the corpus callosum, decreased surface area and volume of the cerebellum, cerebral dysgenesis, hydrocephaly and abnormalities in other cortical and subcortical structures (Archibald et al., 2001; Autti-Ramo et al., 2002; Clarren, 1977; Clarren & Smith,

1978; Coulter, Leech, Schafer, Scheithauer, & Brumback, 1993; Jones & Smith, 1973; Peiffer, Majewski, Fischbach, Bierich, & Volk, 1979; Wisnewski, Dambska, Sher, & Qazi, 1983). Few consistent structural brain defects emerged from autopsy studies, however; the most frequent findings were that of microcephaly and microencephaly (Roebuck, Mattson, & Riley, 1998).

The advancement of non-invasive brain imaging techniques, such as MRI, has greatly contributed to the knowledge of specific structural brain anomalies in individuals prenatally exposed to alcohol. Most MRI studies report small brain volumes in FASD, consistent with autopsy reports (Archibald et al., 2001; Astley et al., 2009; Coles et al., 2011; Roussotte et al., 2012; Sowell et al., 2001). However, these findings are based on group effects, and not every individual with PAE has a smaller brain (Lebel et al., 2011). Nonetheless, because reduction in total brain volume is frequently reported and is a key deficit in FAS, it is often used as a covariate when looking at the volumes of other neuroanatomical structures to determine whether their size differs disproportionately to the overall reduced brain volume (Lebel et al., 2011).

A methodological problem in FASD neuroimaging studies relates to the different criteria used in different diagnostic schemes, which make it difficult to uniformly characterise the structural anomalies in the distinct subgroups of FASD across studies. Despite the diverse methods and assortment of individuals prenatally exposed to alcohol, however, some consistent structural anomalies have been observed.

Aside from total brain volume, white matter and gray matter volumes also appear to be reduced as a result of PAE (Archibald et al., 2001; Bjorkquist, Fryer, Reiss, Mattson, & Riley, 2010; Lebel et al., 2011). The largest white matter tract, the corpus callosum, is especially vulnerable to PAE and has been shown to be affected in variety of ways, ranging

from partial to complete agenesis and malformations (Astley et al., 2009; Clarren et al., 1978; Clark, Li, Conry, Conry, & Loock, 2000; Mattson et al., 1992; Riley et al., 1995), as well as variability in shape, including changes in volume, length and thickness, such as thinning in the anterior and posterior regions (Astley et al., 2009; Bookstein, Sampson, Streissguth, & Connor, 2001; Riley et al., 1995; Sowell et al., 2002; Yang et al., 2012). Investigation of deep gray matter structures has also consistently shown them to have disproportionately smaller volumes (Archibald et al., 2001; Astley et al., 2009; Autti-Ramo et al. 2002; Coles et al., 2011; Cortese et al., 2006; Roussotte et al., 2012). Subcortical structures affected by PAE include the caudate nucleus, hippocampus, (Archibald et al., 2001; Astley et al., 2009; Nardelli, Lebel, Rasmussen, Andrew, & Beaulieu, 2011) and the basal ganglia (Mattson et al., 1994; 1996), including the globus pallidus (Nardelli et al., 2011; Roussotte et al., 2012) and putamen (Astley et al., 2009; Nardelli et al., 2011). Reductions in the volume of the cerebellum have also been demonstrated (Archibald et al., 2001). Similarly, frontal lobe regions and the parietal and temporal lobes have been shown to have less white matter, gray matter and smaller overall lobe volume (Astley et al., 2009; Archibald et al., 2001; Sowell et al., 2002). Alcohol-exposed individuals have also been shown to have a smaller fusiform gyrus (Coles et al., 2011).

The brief summary above demonstrates the extensive and diverse consequences of alcohol-exposure on the development of the prenatal brain. The structural anomalies following PAE are not limited to individuals with the most severe alcohol exposure or diagnosis, but also affect individuals at lower severity levels along the FASD spectrum (Lebel et al., 2011). To better understand the extensive behavioural and cognitive impairments seen in individuals with FASD, it is important to identify the impact these underlying structural abnormalities have on their related neuroanatomical functions. Neuroimaging, specifically

MRI, has allowed us to identify the typical structural composition of the human body (including the brain), as well as study abnormalities associated with, for example, environmental insults, such as PAE, or disease processes. To understand the relation between structural anomalies and observed behavioural/cognitive deficits, we can study the brain's functional activity by means of fMRI.

Investigating the Neurocognitive Profile of FASD Using fMRI

There is a vast body of research documenting the various behavioural and cognitive impairments of individuals with FASD. The advances in the field of neuroimaging have provided the opportunity to investigate the effects of PAE on structural, metabolic and functional changes in the developing brain. Cognitive domains that have been investigated in individuals with FASD using neuroimaging include working memory (Malisza et al., 2005; Astley et al, 2009; O'Hare et al., 2009; Spadoni et al., 2009), associative learning (Sowell et al., 2007), math and number processing (Meintjes et al., 2010; Santjanam, Li, Hu, Lynch, & Coles, 2009; 2012; Woods et al., 2015), inhibitory control (Fryer et al., 2007) and visual attention (Li et al., 2008).

Database searches (using PsycINFO and PubMed) within the FASD and fMRI literature using terms, such as 'affective appraisal', 'emotion recognition', 'affect recognition', 'emotions', 'affect', 'face recognition', found no fMRI study that investigated the cortical activation elicited during affective appraisal in children with FASD. Faces are possibly the most crucial visual stimuli in human social interactions. Intact social and emotional functioning depends on the ability to correctly interpret and respond to social cues, such as the affect displayed on the face (Adolphs, 2010; Calder & Young, 2005). To utilise faces as social cues, these stimuli need to be processed and the affect displayed extracted and linked to an acquired knowledge-base of emotions. Facial affect recognition is, thus, a critical

component determining social behaviour, and difficulties with affect recognition may contribute to social-cognitive impairment. Recognising a face as a face rather than an object and identifying a basic affect/emotion displayed in the face constitute two different processes with different underlying neuronal activations, which involve an array of cortical regions. One could argue though that these two processes resemble a two-step interdependent structure in which first, the face stimulus needs to be recognised as such by identifying and distinguishing the facial features from other stimuli (perceptual processing), after which the configuration of the facial expression is analysed and paired with the knowledge that a certain expression signals a specific emotion (emotion recognition). Face processing, or perceptual processing, involves the regions within the occipital and posterior temporal visual cortices (Adolphs, 2002), as well as lateral parts of the inferior occipital gyrus, fusiform gyrus, and superior temporal gyrus (Allison, Puce & McCarthy, 2000; Haxby, Hoffman, & Gobbini, 2000; 2002).

The process of affective appraisal thus relies on the interrelated functioning of cortical structures forming an emotion-processing network including areas of the visual, limbic and prefrontal cortex (Fairhall & Ishai, 2007; Ishai, 2008; Posmentier & Abdi, 2003). Broadly put, within this network, the visual regions are involved in processing stimuli and extracting the individual features. The visual areas are connected to limbic structures, which project to ventral, medial and dorsal prefrontal regions to where the gathered information is relayed for higher-order processing (Barbour et al., 2010; Kim & Whalen, 2009; Likhtik, Pelletier, Paz, & Paré, 2005).

More specifically, when a face stimulus is presented, the visual information contained in the stimulus is relayed along the occipital and temporal neocortex, and the perceptual information pertaining to faces is extracted. It has been shown that the fusiform gyrus, in particular, is involved with decoding static facial features and thus related to encoding facial

identity. The cortical area surrounding the fusiform gyrus is predominantly activated by the presentation of faces and less so on presentation of other objects including scrambled faces. This region is known as the fusiform face area (Kanwisher, 2000; Kanwisher, McDermott & Chun, 1997). Recently, it has been shown that, in addition to the fusiform face area, a small bilateral site in the anterior tip of the collateral sulcus, is activated during presentation of faces but not of other objects (Nasr & Tootell, 2013). By contrast, the superior temporal gyrus responds to the changeable features of a face and is suggested to be responsible for encoding facial expressions (Haxby et al., 2002). Thus, based on the structural images of the stimulus it is categorised as a face expressing an emotion or not. In addition, the amygdala and orbitofrontal cortices have been shown to be involved in the process that triggers the recognition of the facial emotion displayed. Also, impairments in emotion recognition have been shown to be associated with unilateral and bilateral amygdala damage, and more so for the recognition of social emotions than basic emotions (Adolphs, Baron-Cohen, & Tranel, 2002).

Here, not all structures involved in facial emotion recognition respond equally to all emotions displayed. Thus, the valence of the facial emotion displayed can selectively activate certain regions shown to be involved with facial emotion recognition. For example, neuroimaging studies have shown that the amygdala is disproportionately activated when the subject is presented with fearful facial expressions (Morris et al., 1996; Whalen et al., 2001). Furthermore, the medial prefrontal cortex shows a longer processing time for angry face stimuli compared to happy faces (Harmer, Thilo, Rothwell, & Goodwin, 2001). Also, increased activation in the orbitofrontal and anterior cingulate gyrus (prefrontal cortex) has been demonstrated for angry facial expressions but not for sad facial expressions (Blair, Morris, Frith, Perrett, & Dolan, 1999). However, to date, no neuroimaging studies have

examined the cortico-limbic processing during facial affective processing in individuals with FASD.

Rationale for the Current Research Study

Developmentally, adolescence is a vulnerable period during which cognitive and emotional networks emerge and directly guide our behaviour and decision-making process. According to population studies, facial affect recognition continues to develop into late adolescence (Williams, Mathersul, Palmer, Gur, Gur, & Gordon, 2009). Because human social interactions seem to rely partly on the intact development of cortico-limbic function, neural anomalies or changes in activation patterns may impair the appraisal of social cues resulting in deficits in social behaviour (Adolphs, 2002). Specifically, impairments in the facial emotion processing network involved in affective appraisal of faces may adversely affect the interpretation of affective social cues. Critically, many of the neural regions thought to mediate the cognitive (e.g., WM and attention) and affective processes (e.g., emotion recognition/face processing) necessary for social-cognitive interaction have been implicated in individuals with prenatal alcohol exposure, showing altered neural activations and structural abnormalities. Cortical structures involved in aspects of facial emotion processing that have been shown to be affected by PAE include the orbito-frontal regions, superior temporal lobes, the amygdala and the hippocampus (Adolphs, 2010; Phelps, 2006; Phillips, Drevets, Rauch, & Lane, 2003; Spadoni et al., 2007). Additionally, the general reduction in size in a multitude of cortical regions, as well as the significant reduction in frontal lobe volume in individuals with FASD, may have a significant effect on the overall functioning of the entire facial emotion processing network without singling out specific regions (Astley et al., 2009). Given the social-cognitive deficits seen in children with FASD and the developmental disruption of cortical structures due to the teratogenic effect of alcohol,

alterations in the mechanisms underlying affective appraisal may explain their difficulties in social behaviours. Also, in the first study I showed that children with FASD have difficulties in inferring other's mental states based on the findings on the RME. Investigation of a more basic ability, which is thought to be a precursor to such mentalizing processing, namely the processing of affective social cues, followed in Study II. Here, a valence-related effect was found across groups for affective appraisal confirming that PAE does, indeed, impact the processing of facial affect. This may, in turn, influence the interpretation processes that follow and which ultimately guide the outcome of these individual's social interactions.

Study III Aims and Hypotheses

Study III was designed to identify the brain activity that mediates the effect of PAE underlying the impairments in social cognition reported in the two previous studies. The RME task from Study I was shown to be correlated with the affective appraisal task administered in Study II. The affective appraisal task was deemed suitable for examining differences in cortical activation in an MR scanner because (1) performance of the children on the two tasks (RME and affective appraisal) was significantly related (r=.47, p<.001), (2) the affective appraisal task is easier to administer in the scanner than the RME, and (3) because no significant group differences were found when administering this task behaviourally in Study II. Study III's goal was to be achieved by examining the neural bases of affective appraisal deficits following the administration of the affective appraisal task in the scanner. Given the very high levels of PAE in this population and evidence from cognitive studies that heavily exposed children who perform adequately on simple tasks often process the relevant information using different, probably less optimal neural pathways (Meintjes et al., 2010; Diwadkar et al., 2013**)**, it seems reasonable to hypothesize that children with heavy prenatal

alcohol exposure will show differences in neural activation patterns during affective that are not detectable behaviourally.

Several key questions were formulated to investigate the neural activation patterns in children with FASD engaging in affective appraisal. First, I aimed to establish which cortical regions are activated during affective appraisal across all participant groups, as this is the first study to investigate this domain using neuroimaging in children with FASD compared to typically developing children from the same social environment. For this purpose, the following question was posed: are there differences in cortical activation in school-age children with FASD when performing an affective appraisal task compared to healthy controls? Differences in cortical activation would be confirmed if, for example, a greater recruitment of cortical structures to perform the same task was seen. Given the structural cortical and subcortical anomalies following PAE and the vulnerability of a developmental population, we were interested to establish whether cortical regions of the emotional face processing network were specifically affected by PAE. Hence, if differences in cortical activation are confirmed, are these differences directly related to PAE? Also, how do demographic variables as well as IQ and executive function (EF) either confound or mediate the potential effect of alcohol? Considering the results from study II, where an effect was seen depending on the valence of the affect displayed in the stimuli, it was important to ask: are there different activation patterns depending on the nature of the affect (positive, negative or neutral) displayed? Or, is the potential effect seen independent of valence and only present when comparing face stimuli to non-face stimuli?

Specifically, the predictions for Study III were:

1. There is a difference/variation in the degree to which FAS/PFAS, HE non-syndromal and control children recruit neural networks for affective appraisal

2. There is a difference/variation in the degree to which FAS/PFAS, HE non-syndromal and control children differ in the appraisal of positive, negative and neutral affect.

Methods

Participants

90 children aged 9-14 years from the Cape Town Longitudinal Cohort (Jacobson et al., 2008) were scanned using a Siemens 3T Allegra brain/head MRI scanner (Siemens Medical Systems, Erlangen, Germany; not full body) at the Cape Universities Brain Imaging Centre (CUBIC). As part of the scanning protocol, children had to complete an affective appraisal task while in the scanner. This task had previously been administered behaviourally as a part of a neuropsychological assessment at the UCT CDRL along with a range of intellectual and EF measures (see *Chapter Five*, pp. 103-107, and *Chapter Three*, pp. 44-49, respectively). Based on those behavioural data, which were discussed in the previous chapter, it was determined that all children who were being considered for the fMRI study were able to successfully complete the task. Study II showed that performance on this task was correlated with the RME test. The affective appraisal task is easier than the RME test and, therefore, more suitable for assessing activation differences in this domain in the scanner since none of the participants demonstrated any cognitive or behavioural difficulties impacting their performance on this task. Furthermore, working memory ability did not confound or hinder performance on the affective appraisal task. This task was, therefore, used in the fMRI study to assess cortico-limbic activation during affective appraisal in syndromal and non-syndromal children with PAE.

Exclusion Criteria

Only right-handed participants were included in the functional imaging procedure. Left-handed participants were only scanned structurally. Handedness was assessed prior to the scan using the Edinburgh Handedness Inventory (EHI; Oldfield, 1971), which examines hand preference across a number of domains, such as writing, eating and sports. Handedness is categorised as either right-handed, mixed right-handed, left-handed or mixed left-handed. Any participants falling into either of the left-handed categories were not included in the functional imaging. Excluding left-handers from fMRI studies is a frequent practice because the lateralization of brain structures of left-handers is heterogeneous and potentially different from that of right-handers. Thus, including left-handers in fMRI group comparisons becomes problematic as it adds substantial heterogeneity to the analysis. Assessing handedness using the EHI is common practice in paediatric neuroimaging studies (e.g., Ferrett et al., 2010; White et al., 2013). Furthermore, in adherence with standard neuroimaging safety conventions, participants were not scanned if they (a) had a recent history of surgery, (b) had braces and/or any type of metal inplants in their body, (c) suffer from claustrophobia, or (d) were pregnant. A copy of the safety protocol used at CUBIC is attached in Appendix N. None of the participants needed to be excluded based on these safety criteria, and all children recruited were scanned. Two children recruited as pilot participants were excluded from the analyses.

The behavioural output generated by the participant's button presses during the fMRI was examined to identify any participants whose proportion of correct responses was at or below chance (*d*-prime < 0.15). Three children were excluded based on this criterion; all of the remaining 71 had responses with *d*-prime > 0.25. We further investigated the behavioural data collected in the scanner for any circumstantial or environmental factors that would lead

to the exclusion of the participant, such as, too much movement/motion during image acquisition, which cannot be adjusted for using motion correction, or other problematic/questionable behavioural observations.

Following neuroimaging convention when scanning children, movement parameters were investigated prior to processing the data. Participants with movement greater than 3mm on any of the three movement parameter axes (x, y or z) were excluded from the analyses. See participant flow chart (see Figure 6.5) for details on excluded participants.

Ethical Considerations

For details pertaining to ethical considerations and review board approval common to all studies of this doctoral research, including the fMRI study, see the analogous section in *Chapter Three* (pp. 53-56).

Written informed consent was again obtained from the mothers/primary caregivers on the day of the child's scan in their preferred language, either in English or Afrikaans (see Appendix C and D). Children who were under the age of 10 at the time of the scan gave oral assent, and children aged 13-17 years gave written informed assent (see Appendix E and F).

We employed a non-invasive neuroimaging technique in this study. There are no direct risks associated with fMRI, and participants were extensively briefed prior to their scan. Due to the loud noise the scanner makes during image acquisition, participants were given ear plugs and headphones during the scan to dampen the sound. I administered a standard scanning checklist with the child and guardian to ensure that it was safe to scan the participant (see *Procedure* section below for details). Participants and their caregiver (if they chose to sit in the scanner room with the child) were required to remove any metal items, such as earrings or wrist watches prior to entering the scanner room. Children were thoroughly familiarized with the scanner environment (see *Procedure* section below for details) and only scanned if

they were comfortable with the procedure. I informed them that I would be able to communicate with them throughout the scanning procedure, and their guardian and/or our research nurse sat with them in the scanner room during the entire duration of the data acquisition. The research nurse held a button that she could press to alert the examiner and radiologist to pause/discontinue the scan at the participant's request. They were reminded that they were allowed to discontinue testing at any point should they feel uncomfortable. It should be noted that following the briefing and preparation session, all children were willing to participate further; and all but one participant completed the entire scanning session—this one participant only failed to complete tasks not included in the current investigation. Aside from the standard compensation provided (outlined in *Chapter Three*, pp. 55-56), children received a printed photo of their structural brain scan as a keepsake. The radiologist conducting the fMRI scan examined each participant's structural scan for any gross structural anomalies to determine if follow-up referrals were necessary. None of the scans revealed any such concerns.

Behavioural Task

The affective appraisal task administered in the current study includes face stimuli comprised of normatively rated photographs (Ekman & Oster, 1979) portraying the following basic emotions: happy, sad, angry, fear and neutral. The appraisal of basic or primary emotions is easier to assess than, for example, that of secondary emotions, such as moral emotions (e.g., guilt, shame) because primary emotions, such as fear, sadness and happiness are prototypical and recognizable with the help of universal facial expressions (Ekman, 1993). Furthermore, they are organised in a hierarchical structure in executive operating systems in the brain that act with distinct neural circuits and neurochemicals. Primary emotions appear to

govern higher-level brain functioning such as decision-making processes and thus also influence secondary emotions (Damasio, 1995).

The affective appraisal task used in Study II was adapted for use in the scanner for the current study by adding "distorted" or pixelated images of faces as a control condition (Barbour et al., 2010). The "distorted" images were constructed by taking face stimuli and scrambling them, creating a pixelated effect. For methodological details pertaining to the affective appraisal task, see the *Methods* section of Study II, pp. 103-107. To summarize briefly, photographs of people's faces depicting different emotions (happy, sad, angry, fear, neutral or pixelated/distorted faces) were presented sequentially. During the task, the participant has to decide whether the person they are currently seeing feels the "same" or "different" to the previous person and press one of two corresponding buttons. Participants were not required to label the affect displayed. fMRI activity was assessed for events (i.e., the faces) and contrasted between different participant groups. Following a familiarisation session for both the affective appraisal task and the scanner environment (see *Procedure* section below), the task was administered using fMRI.

Event-related design. Stimuli for the affective appraisal task were programmed using a randomized jittered event-related design (Josephs & Henson, 1999). A jittered design applies different random delays between the start of the acquisition of the brain volume images relative to the start of the stimuli presentation, which allows for different time points to be sampled. The images showing faces (with emotions displaying one of three valence categories; positive, negative or neutral) and the control images (the pixilated/distorted faces) were presented in a pseudo-random order (see Figure 6.3). All stimuli were presented for 2 seconds and randomly jittered with 2-4 seconds (in 0.5 second increments) intervals between stimuli (see Figure 6.4). All data were acquired within a single fMRI session lasting 8 min 12

sec. In total, 96 images (80 affective faces and 16 control images) were displayed continuously during the task with intermittent breaks in the form of fixation crosses between each sequence. These breaks were included to provide participants with the opportunity to rest prior to the next sequence of uninterrupted trials. Overall, for each affective category in the task (happy, negative, neutral), 25% of the trials were target stimuli, (i.e., requiring a 'same' response). For example, a 'same' response, would be required if a 'happy' face was presented following a 'happy' face. A participant would be required to select 'different' as a response if, for example, a 'happy' face was immediately followed by a face displaying an emotion other than 'happy', or by a pixelated/distorted image. If a pixelated face stimuli followed or preceded any affect displaying face the required response would be 'different'. If a pixelated face stimuli was immediately followed by another pixelated face, the response required would be 'same' The pixelated faces provided a control for the perceptual processing of the stimuli, so that the neuronal activations in response to the affective expression the faces could be compared to activations for pixelated image stimuli. During the task, each image was presented only once to avoid repetition effects on the BOLD response (Gilaie-Dotan, Nir, & Malach, 2008).

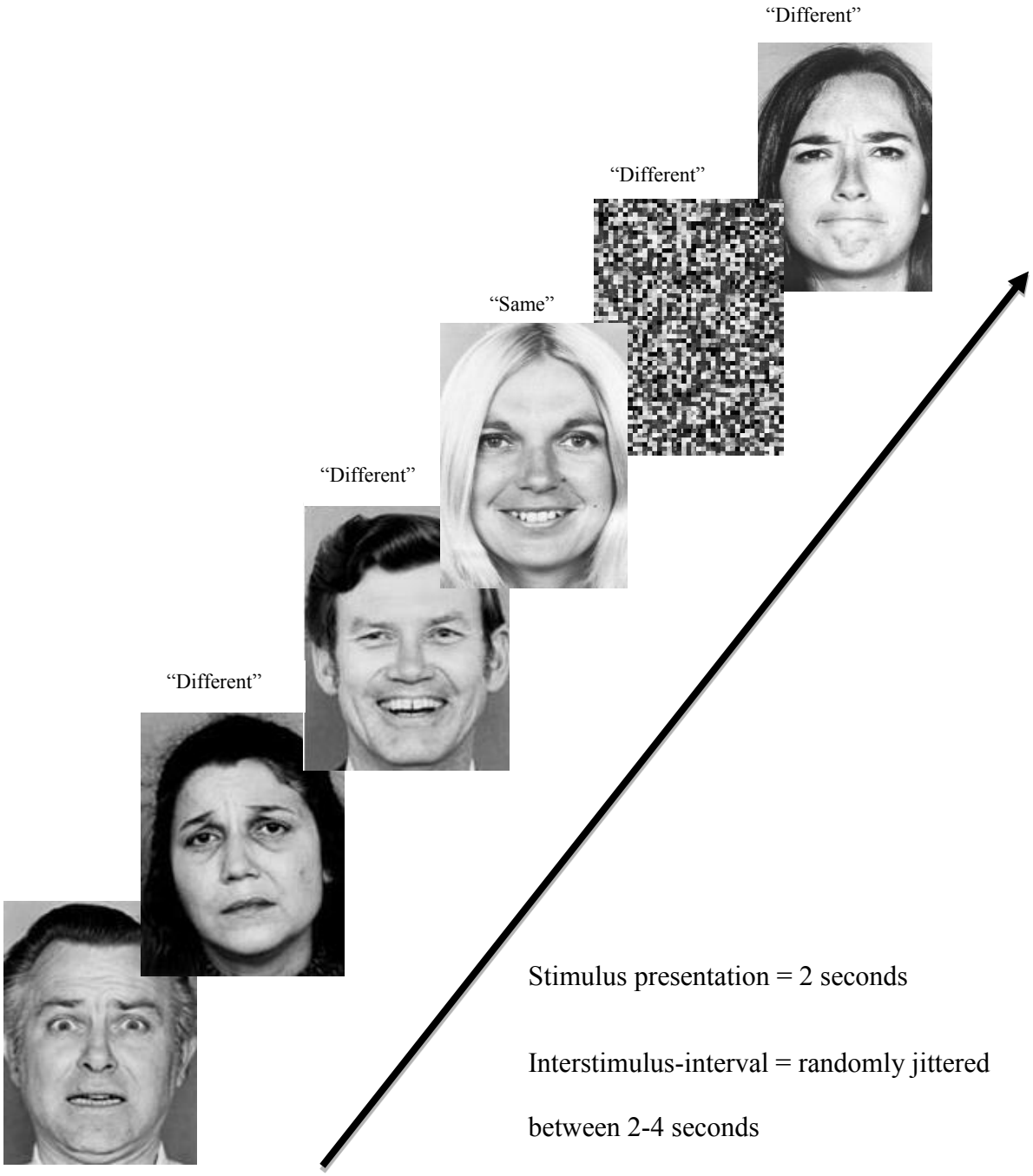

Figure 6. 3. Schematic depiction of the affective appraisal task. The appropriate response to each stimulus is labelled here for exposition purposes only. Details of the task are outlined in the text. Response to each stimulus (face) was assessed behaviourally by pressing one of two buttons on a response box during an fMRI scan. Neuronal activation was measured by BOLD response image acquisition.

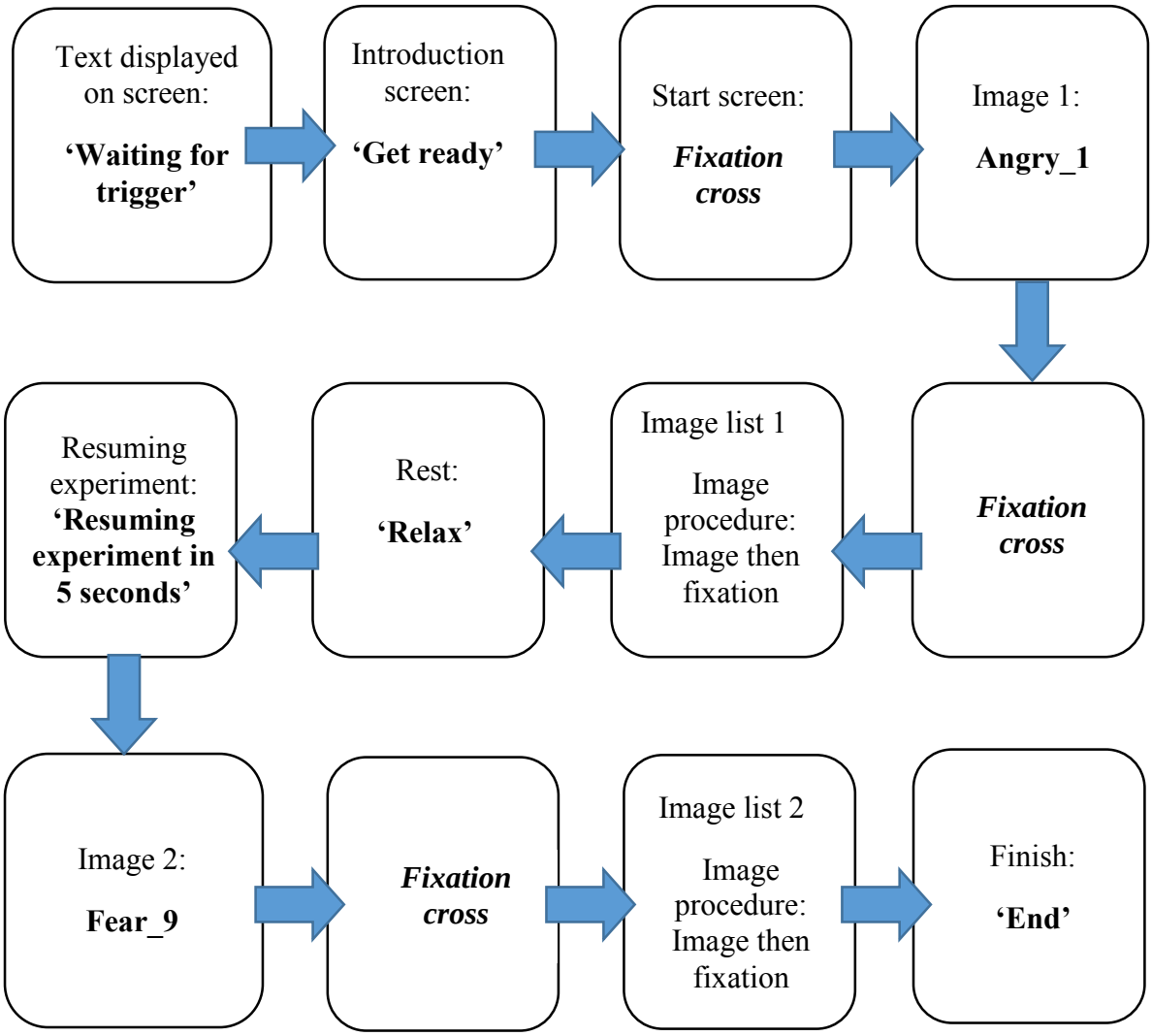

Figure 6.4. Schematic depiction of the continuous affective appraisal task

Procedure

On the day of the scan, the participant and his/her primary caregiver were transported from their home in our research van by our research laboratory's driver and taken to CUBIC where they received breakfast, lunch and a small snack during the morning or lunch and a snack if their assessment was scheduled in the afternoon. Written consent and verbal or written assent were obtained from the primary caregiver and child, respectively (as noted

above). A medical history sheet, provided by CUBIC, was completed to ensure that it was safe for the participant to be scanned (see Appendix N).

Participants were individually briefed and familiarised with the scanning procedures and environment during a mock scanner preparation session (see below). The mock scanner at CUBIC is a simplified, non-functional replica of a MRI scanner to help accustom children to the scanner environment and thereby reduce any potential anxiety, aiding in obtaining high quality MRI scans. At the end of the scan, the mother or primary caregiver received monetary compensation, and the child received a small gift. A printed screenshot of a structural scan of their brain was given to each child within a week of their scan. For a copy of the scanner protocol including directions for participant preparation and the mock scanner session, and instructions given during the scan, see Appendix O.

To avoid experimenter bias, all research assistants including myself were blind to the participant's FASD diagnosis and history of prenatal alcohol exposure and remained blind except in the most severe cases where FAS status was apparent based on the syndromal dysmorphic facial features.

Participant Preparation

A preparation session was run prior to each scan which was important in reducing anxiety and facilitating the completion of the fMRI scans. This session served to (a) familiarize the child with the scanner environment and various sounds of the scanner during data acquisition, (b) explain and practice the tasks that would be administered during the scan, and (c) practice how to react and respond when lying in the scanner still.

Scanner familiarization. Initially, it was explained that the machine would take pictures, much like a camera, but of the inside of their body. We explained that the scanner, which we referred to as 'the big machine' would take pictures of the inside of their head so

that we could see what their brain looks like. We further explained that, because it is quite difficult to take pictures of the inside of someone's body, the machine would take a fairly long time to take all the pictures and would also make loud noises while doing so. Participants were informed that once they got into the scanner, they would first watch a movie for about 15 minutes during which they simply need to lie very still and watch the movie, while the structural scan was done. Participants were allowed to choose one of three movies to watch during this time. Following the movie they would then complete some computer-based tasks while the machine was taking pictures.

Practice of tasks. Aside from familiarizing the participants with the scanner environment, the preparation session provided an opportunity to introduce the participants to the computer-based tasks, including the affective appraisal task that they were to complete in the scanner. All of the children had previously participated in neuroimaging assessments as part of the longitudinal study and had completed a similar version of the affective appraisal task during a visit to the CDRL for Study II the previous year. The scanner preparation sessions provided an opportunity for them to ask questions and practice the new version of the task (which now included the pixelated images) outside of the scanner, ensuring that the task instructions were well understood prior to administration of the task in the scanner. The practice of the affective appraisal task was conducted according to the same guidelines as when administered behaviourally (see Study II, pp. 103-107). Yet, the introduction of the pixelated image and time since the previous administration warranted another practice session. Hence, a paper trial was used initially to practice when to select "same" emotional and when to indicate that it was "different". The child was told not to give a verbal response when performing the task in the scanner, as the head moves when one speaks, which would affect the image quality. For that reason, we explained, that the child needed to press one of

two buttons on a response box instead of giving a verbal response. The response box would rest on their stomach outside of the scanner tunnel, which is why they would be allowed to move their fingers. Participants were instructed to press the button on the left, with their index finger, for when the response was "same" and to press the button on the right, with their middle finger, for when the response was "different". This was practiced a few times on the keyboard before running the paper practice trial again, this time with the participant pressing a button on the keyboard instead of giving a verbal response.

Mock scanner preparation. Given that this study was comprised of a paediatric sample, the practice of the behavioural computer-based tasks was followed with familiarization with the scanner environment. The importance of lying still in order to get good quality pictures was explained, and we practiced this with each child in a mock scanner. A mock coil was placed above their head with a mirror attached in order to familiarize them with how they would be seeing the screen and tasks when lying in the actual scanner. I explained how I would be able to speak to them through an intercom system and that they would hear us through the big headphones placed over their ears. We also explained that we would remind them what they needed to do prior to commencing a new task. The different sounds the scanner makes, while acquiring the fMRI data, were also played on an audiotape and it was explained to the participant that s/he would hear these loud sounds while lying in the mock scanner. We explained to the child that when s/he goes into the actual scanner, the task would work in the same way, except that s/he would be lying down on the scanner bed, press buttons on a response box instead of a keyboard and that the machine would take pictures of the brain while s/he was performing the task. Thus, the task would take a little longer in the scanner than in the practice, as the machine takes a little longer to take all the pictures. At this point we re-assured the child that s/he did not need to worry about sounds the machine makes as the

noise is simply an indication that the machine is busy taking pictures of their brain. We also explained that when they hear the sounds they would then know that the machine is taking pictures and that is when they need to lie very still. Participants could choose whether their mother/primary caregiver would go with them into the scanner room; a research nurse was also always present and sitting next to the scanner bed offering comfort to participant, if necessary.

Neuroimaging Assessment

Data acquisition: fMRI. Each participant was scanned on a 3T Allegra MR scanner (Siemens, Erlangen Germany) at CUBIC. At the beginning of the scanning protocol, a magnetization-prepared rapid gradient echo (MPRAGE) structural (high-resolution T1-weighted anatomical) image was attained in a sagittal orientation with the following parameters: TR = 2530 milliseconds (ms), TE = 1.53 ms, TI = 1100 ms, 128 slices, flip angle 7 degrees, voxel size = 1.3 x 1.0 x 1.3 mm^3, scan time = 8:07 minutes. Each child's structural scan was reviewed by the radiologist to screen for any structural abnormalities. During the affective appraisal fMRI protocol, functional T2-weighted images sensitive to the BOLD contrast signals were acquired using a gradient echo, echo planar sequence [TR = 2000 ms, TE = 30 ms, 34 interleaved slices, 3 mm thick, gap 0.9 mm, field of view 200 x 200mm (in-plane resolution 3.125 x 3.125 mm^2)]. In order to allow the signal to reach steady state, the first 4 volumes were discarded.

The affective appraisal task was programmed and run using E-Prime software (Psychology Software Tools, Inc., Pittsburgh, USA). Stimuli were projected from a computer onto a screen behind the scanner tunnel and viewed through a mirror attached to the coil placed over the participant's head. Participants responded by pressing buttons on a Lumitouch response system (Photon Control Inc., Burnaby, Canada), which is a MRI-compatible

response box. To minimise head movement foam padding was placed around the participant's head. Ear plugs were used to reduce the noise generated by the scanner, and big head phones were placed over the participant's ears to further reduce noise and to allow them to hear the examiner through an intercom system and to listen to sound when watching the movie during acquisition of the structural scan.

Data acquisition: behavioural. The behavioural data compiled of the participant's button-presses during the affective appraisal were analysed to: (a) determine the exclusion of any participants who responded to the task at random, and (b) to ensure there were no significant differences in performance across the three diagnostic groups. The primary behavioural outcome measure of the affective appraisal task was *d*-prime, which measures the number of correct button presses in relation to false alarms (stimulus is 'different', response is 'same'). A negative *d*-prime value indicates that the responses contained more false alarms than hits, which suggests that a participant was either not performing the task properly and was giving random responses or guessing or was simply not able to do the task (irrespective of their performance during Study II). Participants with a *d*-prime value close to zero were also considered to be performing at chance. Based on these criteria, five participants were excluded who either had a negative *d*-prime (n=3) score or a *d*-prime value close to zero (*d*-prime < 0.15; n=2). Reviewing the behavioural notes taken at the scanner confirmed the observation of a problematic performance during administration of either the practice trials outside or during the actual task inside the scanner for these participants and provided additional support for the decision to exclude these five participants from the analyses.

The following three outcome measures characterising the participants' performance were also investigated: (a) a response bias measure, which indicated whether a participant had a tendency to respond 'same' or a bias to respond 'different'; (b) a hit rate, which refers to the

number of hits (stimulus 'same', response 'same') in relation to all responses; and (c) a false alarm rate, which is calculated based on the number of false alarms (stimulus is 'different', response is 'same') in relation to all responses.

Data processing and analyses

Neuroimaging data. Neuroimaging data were analysed using SPM 8 (Statistical Parametric Mapping, Wellcome Department of Imaging and Neuroscience, London, UK). The first four images acquired during each session were excluded from all analyses.

Preprocessing. Prior to commencing with preprocessing all structural scans were manually re-orientated and rotated into the AC-PC plane, and the registration manually checked against the fMRI scans. For each participant, MR images were then preprocessed by applying slice time correction to all 34 slices using an interleaved slice order and slice 17 as the reference slice. As next step, fMRI images were realigned and re-sliced to correct for head movement and then co-registered to the subject's own high resolution anatomical MRI.

Next, considering the potential neuroanatomical differences, including differences in overall brain volume typical for this population, finding and applying the best suited normalisation template was explored to allow for group comparisons (Fonov, Evans, McKinstry, Almli, & Collins 2009; Fonov et al., 2011). Due to morphological changes that occur during brain development, the use of an adult template may not be ideal (Fonov et al., 2011). Thus, in an attempt to find the best possible normalisation template, all images were separately spatially normalised against (a) a standard SPM adult template, (b) a NIHPD (National Institute of Health MRI Study of Normal Brain Development) asymmetric paediatric brain template for 10- to 14-year-olds (both available from the SPM website); and (c) an adapted paediatric NIHPD template created using the template-o-matic toolbox (an add-on for SPM). To create the adapted paediatric NIHPD template, the toolbox utilises the

typically developing paediatric brain templates available from the NIHPD database, which consists of 394 individuals aged 5-18 years. Using this approach, a T1 paediatric template was created by using the age and sex of each participant in the current sample, which is then matched against the NIHPD database. On inspection it was found that none of the three templates fit the images in a way that was clearly preferable, none were better or worse. It was, therefore, decided that the adapted paediatric NIHPD template would be used because it is the only template that takes demographic details of the sample into consideration.

As a final preprocessing step, the images were smoothed spatially by a Gaussian filter of 5mm full-width half maximum (FWHM) to improve inter-subject comparability and to reduce noise. Lastly, the default masking threshold in SPM was lowered from 0.8 to 0.7.

Onset time calculation. Prior to first - and second level analysis the event onset times for each child were extracted, i.e., the exact time each stimulus was presented. For these calculations the behavioural data of the AA task was exported from the E-prime data files into an MS Excel spreadsheet. Because the 96 images were presented in a pseudo-random order, the onset time differed for each participant and needed to be calculated. E-prime records the following times (in milliseconds): start time of the task (the time the introduction screen is presented), the start onset time (the time when the task actually starts), and the image onset time (i.e., the time when each image was presented). To match the neural activation signal to the presented image, the different time points need to be converted. This is an important process in the analysis of event-related data, as the hemodynamic response only occurs over a very short and specific time period (Huettel, Song, & McCarthy, 2008).

For this calculation, the time taken to acquire the first four images, which were subsequently discarded, was subtracted from the image onset time, which was then converted to seconds (the time format used by SPM). The task was programmed to be triggered

automatically by the scanner after 4 pulses, lasting 2 seconds each. The pulse on which the task was triggered was recorded for each participant because due to a scanner error, the trigger varied for some participants. If the task triggered with the 4th pulse as it should, no time adjustments were made to the calculated onset times. If the task did not trigger on the 4th pulse, depending on which pulse the task triggered on, 2 seconds per pulse were either added or subtracted to the calculated onset time. Hence, the following adjustments were made depending on when the task triggered: 2nd pulse, 4 seconds were subtracted; 3rd pulse, 2 seconds were subtracted; 4th pulse, 0 seconds were added; 5th pulse, 2 seconds were added; 6th pulse, 4 seconds were added; 7th pulse, 6 seconds were added. These final onset times were then organised according to the image displayed (happy, sad, fear, angry, neutral or distorted) and entered into SPM during first level analysis.

First-level analysis. During the first level analysis, activation maps were generated for each participant using general linear model analysis (GLM; Amaro & Barker, 2006). Each of the 6 image conditions (happy, sad, angry, fear, neutral and distorted) was set up as an individual regressor and the negative emotions (sad, angry and fear) were further combined as a 7th regressor, presenting a negative emotion group. During the model specification step, the image condition, its previously calculated onset times, and the duration of presentation of each image were entered for each participant.

To isolate the differently valenced emotions expressed in the stimuli conditions and to set up the initial contrasts of interest, the following *t*-contrasts were defined for each participant using a contrast manager, where '>' refers to greater activation in a certain region for the first condition, compared to the second: (1) All faces > Distorted Faces; (2) Positive faces > Distorted Faces; (3) Negative faces > Distorted Faces; and (4) Neutral faces >

Distorted Faces. 'All faces' refers to all non-distorted faces, i.e., every stimuli displaying any emotion.

We were also interested in examining contrasting emotions with each other and not just to the distorted images because a specific valence of the emotion may reveal further differences in regional activations related to valence itself. Neutral faces are difficult to interpret as they do not display an obvious positive or obvious negative emotion and thus may require more effort to categorise and recruitment of more brain regions. It was, therefore, decided that negative emotions would be contrasted with the positive (happy) emotions, which are considered the easiest to recognise. Given that in Study II, which examined affective appraisal behaviourally, some group differences were specifically found on the 'angry' emotion, the negative emotions were also contrasted with "happy" individually, rather than as a group. Thus, the following additional contrasts of interests were set up: (7) Angry > Positive; (8) Fear > Positive; (9) Sad > Positive; (10) Positive > Angry; (11) Positive > Fear; and (12) Positive > Sad.

Second-level analysis. Second-level analysis was aimed at identifying clusters of significant activation within and between three groups of children: children with FAS and PFAS[2], HE nonsyndromal children and controls. Both within and between group differences were examined based on the images and contrasts generated at the first-level. Initially, within-group activations were examined for the first contrast (All faces > distorted) to determine whether the regions involved in facial processing are activated more when looking at faces in

[2]Participants diagnosed with FAS and PFAS were subsequently grouped together, and their performance was analysed as one group referred to as the FAS/PFAS group. These participants were grouped together rather than separately because (1) of the smaller number of participants with full FAS and (2) to simplify the matrix design for the fMRI analysis, where three groups is less complex than four.

comparison to the distorted (control) images. This analysis indicates that each group of participants is recognising and processing the faces as such and also a basis for determining which regions the controls, i.e., typically developing children activate when processing these affective stimuli. This was done by using a fixed-effects analysis by running one sample *t*-tests comparing the BOLD signal during the presentation of faces displaying an affect (i.e., experimental condition) and distorted images (i.e., control condition) for both the alcohol-exposed (FAS/PFAS and HE groups) and control groups separately.

In a second comparison, the three emotion groups (positive, negative and neutral) were contrasted with the distorted images to investigate whether there were differences in activation depending on the valence of the emotional stimuli within a specific group. Next, these contrasts were compared between the three groups to examine potential differences in neural activation across the groups using independent samples *t*-tests. Regional activation was then examined for within- and between-group differences for the remaining stimuli contrasts (i.e., the neutral and individual negative emotions vs. "happy").

First, a whole-brain analysis was conducted to establish whether within each participant group the cortical regions involved in face perception were activated. This was done by grouping together all face stimuli, independent of the emotional valence displayed, and contrasting them to the distorted images (non-face stimuli). This comparison was aimed at establishing that all participants process the face stimuli as faces and are able to differentiate them from non-face stimuli, a pre-requisite before then processing the affect displayed. Second, and following the initial whole-brain analysis, a 'Regions of Interest' (ROI) analysis was conducted. For this analysis, anatomically defined regions were selected based on their involvement in face- and facial emotion processing. The cortical and subcortical structures defined as the ROI network used for the affective appraisal task were:

the primary visual cortex, fusiform gyrus, amygdala, ventral prefrontal cortex, dorsal prefrontal cortex and the basal ganglia (putamen and caudate). The ROI analysis was aimed at establishing potential group differences in activation within the facial-emotion processing network, in order to characterise the effect of PAE on the underlying neural processes of affective appraisal ability. When comparing the contrasts, regional activations were examined at a cluster level corrected *p*-value of ($p_c < .05$) to detect minimum extent thresholds of contiguous voxels and, thus, to identify significant clusters (Ward, 2000). To control for false positive rates when doing multiple comparisons 3dClustSim (http://afni.nimh.nih.gov), a widely used cluster correction procedure based on Monte Carlo simulation was employed. Images are statistically contrasted at the group level with respect to number of voxels, the voxelwise statistical values and spatial smoothness to estimate the probability of observing a false positive. During this process, each region forming part of the defined ROI network was examined separately to define the voxel activation intensity and cluster extent thresholds, which were then used to determine a minimum cluster size for each region. What remained are clusters within each region that survive at a corrected significance of $p<0.05$ with a voxelwise threshold of $p<0.05$. All the individual cluster corrected regions were then combined using SPM to generate a cluster-size corrected ROI network.

Statistical analysis

A control variable was treated as a potential confounder if it was correlated with an outcome at $p<.10$. From the comprehensive set of demographic variables collected, the following four control variables were identified as potential confounders of the effect of prenatal alcohol exposure: child's sex and age at scan, mother's education and smoking during pregnancy. Analysis of covariance was run to determine whether the effect of alcohol remained significant after control of the potential confounders.

To get a better understanding of the activations during the affective appraisal task, intra-group 1-sample *t* tests were run for each group first. Secondly, independent sample *t*-tests were run to explore inter-group differences. Finally, to examine whether significant effects were related to the effect of alcohol, the main contrasts were then re-run to control for potential confounding demographic variables, namely maternal smoking, maternal education, child sex and age. These variables were entered simultaneously at the second-level SPM analysis as four covariates. Similarly, measures of participant's IQ and four domains of EF were analysed separately as potential mediators of the effect of alcohol in a second-level SPM analysis separate from confounders.

Results

The administration of the affective appraisal task generated both behavioural and neuroimaging data. The behavioural data were analysed to determine whether all participants were able to discriminate between the affective stimuli and whether there were group differences in performance between the three diagnostic groups (FAS/PFAS, HE and controls). Neuronal activity during fMRI was investigated next by running within- and between-group analyses across the three diagnostic groups (FAS/PFAS, HE and controls) for each of the specified ROIs.

Sample Characteristics

A participant flow diagram (see Figure 6.5) displays the progression of the participants through each phase of the study and the reasons for exclusion. Of the 90 participants scanned, data from 64 children (18 FAS/PFAS; 18 HE and 28 Controls) were included in the final analyses. Demographic and background characteristics of the participants are summarised in Table 6.1.

The continuous alcohol measures collected prospectively during three prenatal visits provided information on the maternal drinking patterns of this cohort and its relation to FASD diagnosis. Alcohol consumption during pregnancy was measured in absolute alcohol (AA; see *Methods* section in *Chapter Three*, pp. 40-41). There was a significant between diagnostic group difference for AA/day, AA/drinking day and proportion of drinking days across pregnancy. As anticipated, mothers of children with FAS/PFAS and HE drank significantly more than mothers of control children (all *p*s<0.001), whereas the amount of alcohol consumed did not differ significantly between the two exposed groups (all *p*s>0.05). Mothers of exposed children concentrated their drinking and drank on average 7-9 standard drinks over 1-2 days/week. All of the mothers in the control group abstained from drinking. Two-thirds of the women reported smoking during pregnancy with the FAS/PFAS and HE mothers smoking significantly more than the control group (all *p*s<0.05). At the time of recruitment, drug use other than smoking and alcohol was very low: 7 women (10.93%) reported using marijuana (2 women 1-2 days/month; 3 women 3-4 days/month and 1 woman 7 days/month); none used cocaine. Because prenatal exposure to these drugs were too rare for statistical adjustment, associations with prenatal alcohol use were rerun omitting the children exposed to these drugs.

As part of the behavioural assessment during Study I, IQ and EF tests were also administered. The data from that assessment are used in this study to examine the potential mediation of IQ and EF on the effect of alcohol. For more details on the cognitive tasks used here and administered in Study I see *Chapter Three* (pp. 43-49).

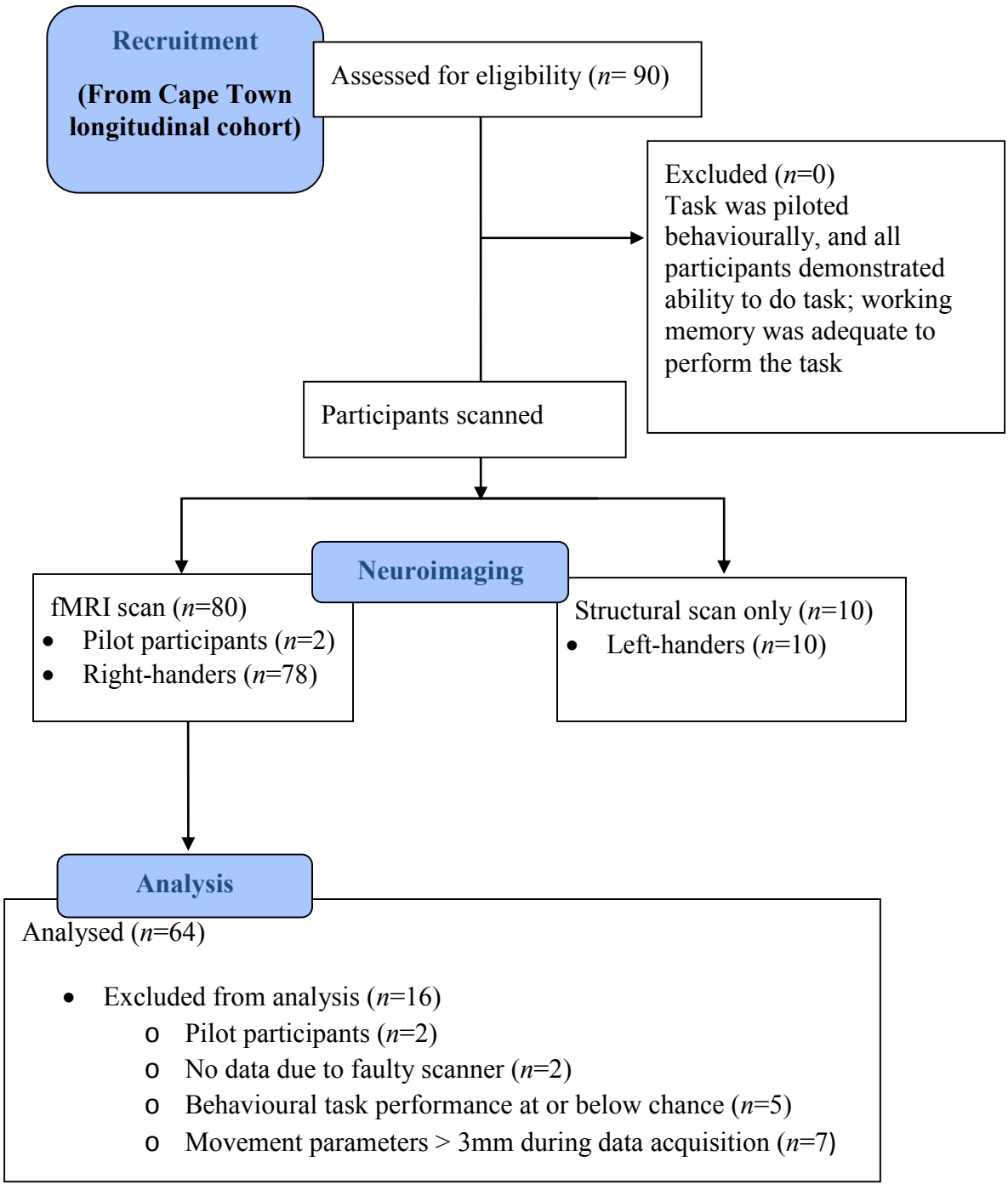

Figure 6.5. Participant flow diagram

Table 6.1.

Sample Characteristics

	FAS/PFAS (*n*=18)	HE (*n*=18)	Controls (*n*=28)	*F* or χ^2	*p*
Maternal characteristics					
Age at delivery	29.3 (6.7)	25.8 (4.8)	25.6 (5.4)	2.75	0.072
Education	8.89 (2.03)	9.33 (2.72)	10.32 (1.85)	2.63	0.080
Alcohol use during pregnancy[a]					
AA/day (oz)	1.0 (0.7)	1.0 (1.0)	0.0 (0.0)	18.68	<0.001
AA/occasion (oz)	4.1 (1.5)	4.0 (3.6)	0.0 (0.0)	30.07	<0.001
Frequency (days/week)	1.6 (0.8)	1.3 (1.0)	0.0 (0.0)	39.70	<0.001
Smoking during pregnancy (cigarettes/day)	7.0 (5.9)	5.7 (4.2)	2.7 (4.6)	4.89	0.011
Child characteristics					
Age at scan	13.1 (1.2)	11.6 (0.7)	11.9 (1.0)	13.50	<0.001
Sex (% male)	55.6	50.0	39.3	1.26	0.532
WISC-IV IQ[b]	64.6 (9.2)	78.1 (17.8)	79.3 (13.5)	6.88	0.002
Executive function					
Attentional Control[c]	0.8 (1.4)	-0.8 (1.1)	-0.3 (0.9)	9.06	<0.001
Cognitive Flexibility[c]	-0.05 (1.3)	0.09 (1.6)	-0.01 (1.2)	0.05	0.956
Information Processing[c]	-1.6 (0.9)	1.0 (2.1)	0.4 (1.6)	11.88	<0.001
Goal Setting[d]	2.5 (1.1)	2.4 (1.2)	2.9 (1.0)	1.25	0.294

Note. Values are mean (SD) or %. [a]Estimated for mothers of children with FAS who denied drinking by taking the mean of the other mothers of children with FAS. [b]Wechsler Intelligence Scale for Children, Fourth edition. [c]Standard scores; Attentional control: FAS/PFAS=18, HE=13, Controls=22, Cognitive Flexiblity and Information Processing: FAS/PFAS=17; HE=18; Controls=27; [d]Raw scores; Goal Setting: FAS/PFAS=17, HE=18, Controls=26.

Behavioural Results

Behavioural results from the scanner (Table 6.2) were analysed to determine whether performance on the affective appraisal task was comparable across the diagnostic groups. Participants with a negative *d*-prime value or a value close to zero were excluded from the analysis (*n*=5). No significant differences were found between groups for Hit Rate, $F_{2,61}$ =1.45, p>.05, η^2=0.045) or False Alarm Rate, $F(2,61)$=0.27, p>.05, η^2=0.009). The discrimination performance for the final sample (*n*=71) was assessed using analysis of variance. No between-group differences were observed on *d*-prime, F(2,61)=1.00, p>.05, η^2=0.032). Differences in response bias were also examined. A response bias demonstrates whether a participant responds preferentially either "same" or "different". No significant group differences were found, $F(2,6)1$=1.26, p>.05, η^2=0.040) except that all groups tended to show a slight preference to respond "different" (FAS/PFAS: mean = 0.65, SD = 0.50; HE: mean = 0.57, SD = 0.51; Controls: mean = 0.44, SD = 0.37). The analysis of the behavioural data showing comparable performance by the diagnostic groups, therefore, indicates that the investigation of neuronal activity can be conducted without having to consider the impact of group differences in performance on the task. Four variables were considered as potential confounders and correlated with the AA outcome measures, all ps > 0.01, and were thus not further considered.

Table 6.2.

Behavioural Data of Affective Appraisal task outcome during the scan

	FAS/PFAS *n*=18		HE *n*=18		Controls *n*=28			
	M	SD	*M*	SD	*M*	SD	*F*	*p*
Inside scanner								
d-prime	1.03	0.55	1.23	0.52	1.25	0.55	1.00	0.374
Hit rate	0.46	0.22	0.52	0.24	0.56	0.17	1.45	0.242
False alarm rate	0.15	0.13	0.14	0.08	0.16	0.10	0.27	0.764
Response bias	.65	0.50	0.57	0.51	0.44	0.37	1.26	0.290

Neuroimaging Findings

The neuroimaging findings are based on the neuronal activity within the regions of interest network following the various steps of data processing and cluster size correction, outlined above. As an initial exploration, we investigated intragroup activations for each group to explore which regions of the network were activated within each group. As the next step, we examined group differences across various contrasts and conditions. The results tables below summarise the brain regions with greater activation using the Montreal Neurological Institute (MNI) co-ordinate system, number of voxels (i.e., the size of the cluster) and the *t*-value of the peak within the activated cluster.

Whole-brain analysis. We first examined activations based on a whole brain analysis rather than a ROI analysis. Table 3 lists regions showing greater activity when looking at the different valenced faces (all, positive, negative and neutral) in contrast to the distorted faces (control images) within each group. The distorted image is often used as the reference image as it gives a pure visual baseline and is thus used as control condition. This analysis provided an

initial activation overview for each group to see whether there are regions that are activated more when looking at faces in general (irrespective of affect displayed) versus distorted images. Table 6.3 summarises the regional activations using extent thresholds of contiguous voxels ($p<.01$) before cluster-size correction but at corrected $p(\text{unc}) < .05$. At this selected threshold, the control and HE groups activate more regions during face processing compared to the FAS/PFAS group. Similarly, most regional overlap is between the control and HE groups. This initial investigation confirmed a basic question, namely that participants did indeed process the face stimuli differently from the distorted facial stimuli. Given the increased activation for faces versus distorted images, it was then warranted to continue with a more in-depth and directional ROI analysis.

Table 6.3.

Whole-brain analysis showing regions with greater activation for faces compared to distorted images (threshold p<.01, cluster sizes of 5.3 – 59 voxels, voxel size = 2x2x2)

Region	MNI coordinates	Number of voxels	Peak *t*
Control group (*p*(unc) < 0.05)			
Parietal			
R parietal lobule; inferior	42, -50,54	774	3.93
Frontal			
L precentral gyrus	-14, -100,8	2085	6.57
L frontal gyrus; medial	-34,-14,62	2013	6.75
L frontal gyrus; middle	-40,-30,52		4.19
R frontal gyrus; middle	44,-50,-18	400	4.50
R cingulate gyrus	32,-48,-26		3.60
Occipital			
R cuneus	34,14,66	232	4.23
R postcentral gyrus (parietal)	30,-10,60		3.62
Limbic			
L cingulate gyrus	-6,2,54	1255	4.40
L cuneus (occipital)	-8,10,46		4.24
R lingual gyrus (occipital)	12,22,30		3.99
FAS/PFAS group (*p*(unc) < 0.05)			
Parietal			
R sub-gyral	32,24,6	322	4.02
Frontal			
R precentral gyrus	12,-98,12	2196	6.25
Occipital			
R occipital gyrus; middle	30,-30,44	278	4.09
R lingual gyrus	26,-22,44		3.24
HE group (*p*(unc) < 0.05)			
Parietal			
R postcentral gyrus	26,4,48	201	3.05
Frontal			
L cingulate gyrus	-18,-80,-12	1101	4.74
R frontal gyrus, medial	6,16,46	1657	5.59
L precentral gyrus	-2,20,40		4.68
L frontal gyrus; inferior	-4,16,48		4.61
L frontal gyrus; middle	-40,-22,60	1713	5.56
L sub-gyral	-36,14,-2	358	3.78
R frontal gyrus, middle	42,26,30	279	4.40
R frontal sub-gyral	46,-4,26	359	3.97
R cingulate gyrus (limbic)	56,4,28		3.50
Occipital			
R cuneus	30,-2,56	201	3.90
R lingual gyrus	30,-4,64		3.29
Sub-cortical			
L thalamus; ventral lateral nucleus	-42,-6,24	277	3.63

L insula	-26,-8,34		2.97
Sub-lobar			
R claustrum	42,-52,-16	212	3.55
R fusiform gyrus (temporal)	44,-40,-16		3.54

Note. Submaxima within clusters are shown under maxima in italics.

Regions of interest analysis. ROI analyses were run based on the face processing network (see *Methods* section, p. 160), contrasting all faces, independent of the valence of the affect displayed, against the distorted images. Only activations for cluster size corrected regions within the ROI network were compared within and between the three participant groups. Table 6.4 summarises the activation maps for within group comparisons. Numerous brain regions within the ROI network showed significantly greater activation for faces than for distorted images. For control participants, the largest clusters that showed an increased activation (for faces compared to distorted for images) included regions within the right and left fusiform gyrus, right lingual gyrus, right and left anterior cingulate, left cingulate gyrus and right frontal gyrus. Similarly to controls, the FAS/PFAS group appears to recruit the left and right fusiform gyrus, right and left lingual gyrus and the right inferior and middle frontal gyrus. The largest clusters with increased activation in the HE group were consistent with regions activated by both the control group (right fusiform gyrus, left cingulate gyrus, right frontal gyrus) and the FAS/PFAS group (right inferior gyrus). The HE group also showed activation in larger clusters in other frontal regions compared to the other two groups, namely in the right medial frontal gyrus and left inferior frontal gyrus. It is of particular interest that, within each group, the fusiform gyrus showed greater activation when looking at faces in contrast to distorted (non-face) images. The fusiform gyrus has been shown to be involved in face recognition (Adolphs, 2002). These findings, therefore, confirm that each group independently processes and recognises the face stimuli as faces.

Table 6.4.

Within-group comparison of regions of interest network showing greater activation for faces compared to distorted images after cluster size correction (threshold p<.05; cluster sizes of 8.3 – 107.2 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group			
Frontal			
R frontal gyrus, inferior	48,2,34	161	2.98
R frontal gyrus, middle	38,2,38		2.71
L frontal gyrus, middle	-38,26,34	88	3.20
Temporal			
R fusiform gyrus	44,-50,-18	428	4.50
L fusiform gyrus	-38,-46,-20	203	3.72
Occipital			
R lingual gyrus	14,-92,-4	394	6.10
R sub-gyral	36,-62,-12	428	2.57
L cuneus	-14,-96,0	394	4.92
Limbic			
R anterior cingulate	10,24,28	420	3.95
L anterior cingulate	-6,28,28		3.40
L cingulate gyrus	-6,24,36		3.38
Lentiform nucleus			
R putamen	22,14,0	80	2.87
FAS/PFAS group			
Frontal			
R frontal gyrus, inferior	44,4,32	228	2.85
R frontal gyrus, middle	42,56,-8	209	2.31
L frontal gyrus, inferior	-40,50,4	69	2.42
L frontal gyrus, middle	-36,60,4		2.00
L precentral gyrus	-56,2,32	77	2.84
Temporal			
L fusiform gyrus	-36,-58,-16	66	2.75
Occipital			
R lingual gyrus	14,-92,-6	476	5.78
L lingual gyrus	-14,-92,-6		4.41
R fusiform gyrus	28,-78,-18	379	3.18
Lentiform nucleus			
R putamen	18,14,2	128	2.49
R extra-nuclear	30,4,-2	208	2.83
HE group			
Frontal			
R frontal gyrus, inferior	56,4,28	267	3.50

R precentral gyrus	46,-4,26		3.98
R frontal gyrus, medial	10,30,34	334	4.26
R frontal gyrus, middle	42,28,34	90	3.38
L frontal gyrus, inferior	-46,2,36	270	2.95
L precentral gyrus	-42,-6,24		3.63
L frontal gyrus, middle	-44,26,34	125	3.48
Temporal			
R fusiform gyrus	42,-52,-16	318	3.56
Occipital			
L cuneus	-8,-94,0	277	4.14
R cuneus	14,-96,0		3.00
Limbic			
R parahippocampal gyrus	28,2,-20	17	2.37
L cingulate gyrus	-12,24,34	334	2.70
Sub-lobar			
R putamen	22,10,4	301	2.90
R extra-nuclear	26,8,12		2.82
L putamen	-20,12,0	153	3.13
L extra-nuclear	-26,12,-6		2.60

Note. Submaxima within clusters are shown under maxima in italics.

Table 6.5 lists the regions showing significant between-group differences for the three participant groups. When comparing differences in activation between participant groups, the FAS/PFAS group shows increased activity in more regions within the network compared to the control groups. For example, the FAS/PFAS group shows greater activation than controls in the left and right fusiform gyrus, frontal gyri and in sub-cortical structures, such as in the left and right lateral globus pallidus, the right caudate body and bilaterally in the putamen. By contrast, the control participants do not show greater activation in any regions when compared with the FAS/PFAS group. Similarly, the HE group shows greater activation in more regions when compared to the control group but in fewer regions than the FAS/PFAS group. In contrast to the control group, the HE group also shows increased activation in sub-cortical structures, such as the left lateral globus pallidus and left putamen. Very few greater activations are shown for comparisons between the control and HE groups and between the FAS/PFAS and HE groups.

Relatively few regions show greater activations for the HE group compared with the controls and for the HE group compared with the FAS/PFAS group. The between-group differences in activation pattern reflect degree of severity of prenatal exposure, with the FAS/PFAS group

Table 6.5.

Between-group comparison of regions of interest network showing greater activation for faces compared to distorted images after cluster size correction (threshold p<.05; cluster sizes of 8.9 – 127.3 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group	No significant differences		
Control group > HE group			
Occipital			
R cuneus	20,-94,0	44	3.68
Limbic			
R parahippocampal gyrus	26,-10,-18	86	3.01
R extra-nuclear	22,-12,-10		2.54
FAS/PFAS group > Control group			
Frontal			
R frontal gyrus, inferior	54,14,28	59	2.53
R frontal gyrus, middle	44,54,10	65	2.68
L frontal gyrus, inferior	-50,4,30	42	1.83
L subgyral	-36,6,28		2.64
L frontal gyrus, middle	-44,48,8	53	2.80
Temporal			
R fusiform gyrus,	36,-40,-12	76	2.21
extending to occipital lobe	30,-56,-12		2.61
L fusiform gyrus	-28,-34,-20	51	2.00
Occipital			
R fusiform gyrus			
Sub-lobar			
R globus pallidus, lateral	18,-2,8	170	3.20
R putamen	28,0,10		2.33
R extra-nuclear	22,-12,10		2.06
L globus pallidus, lateral	-26,-18,0	107	3.01
L putamen	-26,-8,12	109	2.57
L extra-nuclear	-22,-2,16		2.13
R caudaute body	14,8,18	49	2.79
FAS/PFAS group > HE group			
Frontal			
R frontal gyrus, inferior	50,26,22	24	2.34

R fronal gyrus, middle	48,52,0	120	2.54
Temporal			
R fusiform gyrus	56,-4,-30	101	3.21
Occipital			
R cuneus	20,-94,0	86	3.52
HE group > Control group			
Frontal			
L frontal gyrus, inferior	-46,4,36	143	2.61
L frontal gyrus, middle	-36,12,34		2.27
L precentral gyrus	-42,-6,24		3.31
Limbic			
R parahippocampal gyrus	28,2,-18	26	3.05
Sub-lobar			
L globus pallidus, lateral	-22,-14,4	226	3.87
L extra-nuclear	-32,-22,-2		2.48
L putamen	-24,-10,12		3.44
R extra-nuclear	8,0,4	27	2.53
HE group > FAS/PFAS group			
Frontal			
R frontal gyrus, middle	38,30,34	48	2.71

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

showing the most extensive activations and the HE group intermediate between the FAS/PFAS and control groups.

As the next step in the analysis process, potential between-group differences in activation (within the ROI network) were examined based on the valence of the affect displayed in the stimuli. Positive, negative and neutral emotion conditions were each contrasted against the distorted images. For positive faces contrasted with distorted images, the control group did not show greater increased activation for any regions in comparison to both the FAS/PFAS and HE groups (Table 6.6). In contrast, both the FAS/PFAS and HE groups showed greater increased activation compared to the control group, with the FAS/PFAS group activating more regions than the HE group compared to controls. Differences in regional activation were also seen when comparing the FAS/PFAS and HE groups. Amongst other differences, in comparison to both the

HE and control group, the FAS/PFAS group showed greater activation in the right fusiform gyrus, right lingual gyrus and right middle frontal gyrus. By contrast, the HE group showed greater activation in the right and left frontal inferior gyrus and right precentral gyrus regions in comparison to the FAS/PFAS group.

For the negative faces, the FAS/PFAS did not activate more regions in comparison to the control group and activated only one small cluster more strongly than the HE group (Table 6.7). Both the control and HE groups activated more regions when compared to the FAS/PFAS group, including in the left fusiform gyrus and left cuneus.

For the neutral faces, neither the control group nor the HE group activated any regions more strongly than the FAS/PFAS group (Table 6.8). However, the FAS/PFAS group showed increased activation compared to both the HE and control group, including in the right fusiform gyrus, left frontal middle gyrus, right frontal middle gyrus, left lingual gyrus and bilaterally extra-nuclear sub-lobar regions.

Table 6.6.

Between group comparison of regions of interest network showing greater activation for positive faces compared to distorted images after cluster size correction (threshold p<.05; cluster sizes of 8.2 – 93.4, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group	No significant differences		
Control group > HE group	No significant differences		
FAS/PFAS group > Control group			
Frontal			
R frontal gyrus, inferior	46,14,26	214	3.06
R frontal gyrus, middle	34,20,34		2.99
L frontal gyrus, inferior	-50,4,32	148	2.81
L frontal gyrus, middle	-48,48,10	92	3.07
Occipital			
R lingual gyrus	2,-86,-2	45	3.52
R fusiform gyrus	30,-56,-12	63	3.00
Temporal			
R sub-gyral	38,-30,-8	28	3.07
extending to R extra-nuclear	32,-24,-6		2.02
Sub-lobar			
L caudate-head	-14,22,4	214	3.02
FAS/PFAS group > HE group			
Frontal			
R frontal gyrus, middle	48,26,24	17	2.73
Occipital			
R fusiform gyrus	28,-56,-12	29	2.82
R cuneus	8,-84,4	67	2.89
R lingual gyrus	22,-86,0	41	3.10
HE group > Control group			
Frontal			
R frontal gyrus, superior	24,60,-6	196	4.36
R frontal gyrus, middle	34,60,-8		2.66
R frontal gyrus, medial	14,62,4		2.50
R precentral gyrus	60,2,30	333	3.05
R frontal gyrus, inferior	54,14,28		2.81
L frontal gyrus, superior	-30,66,4	395	2.96
L frontal gyrus, middle	-36,16,34	195	2.55
Temporal			

L Sub-gyral	-34,-36,2	33	2.32	
Limbic				
L anterior cingulate	-12,22,26	90	2.38	
Sub-lobar				
R extra-nuclear	20,-12,26	60	3.00	
L extra-nuclear	-14,24,4	67	2.19	
HE group > FAS/PFAS group				
Frontal				
R frontal gyrus, inferior	42,36,16	160	3.57	
R frontal gyrus, middle	34,44,16		2.50	
R precentral gyrus	62,4,30	68	3.02	
L frontal gyrus, inferior	-36,34,16	82	2.51	*Note.*
L frontal gyrus, middle	-40,44,14		2.29	
Sub-lobar				
R extra-nuclear	18,-4,22	29	1.82	

Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

Table 6.7.

Between group comparison of regions of interest network showing greater activation for Negative faces compared to distorted images after cluster size correction (threshold p<.05; cluster sizes of 9.1 – 119.2 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group			
Frontal			
L frontal gyrus, medial	-4,48,42	80	2.25
Temporal			
L fusiform gyrus	-46,-8,-28	151	3.17
Occipital			
L fusiform gyrus	-36,-46,-12	112	2.67
L cuneus	-16,-90,4	33	2.61
Limbic			
L anterior cingulate	-4,32,10	116	2.50
Sub-lobar			
R extra-nuclear	18,14,18	232	2.86
R putamen	20,8,10		2.62
Control group > HE group			
Frontal			
R frontal gyrus, medial	2,32,-16	15	2.35
Occipital			
R cuneus	12,-94,0	52	2.45
R lingual gyrus	20,-86,-2		2.01
Sub-lobar			
R caudate-head	6,14,-4	80	3.43
FAS/PFAS group > Control group	no significant differences		
FAS/PFAS group > HE group			
Occipital, R cuneus	10,-84,4	35	2.56
HE group > Control group			
Frontal			
R frontal gyrus, superior	22,60,-6	80	3.38
R frontal gyrus, inferior	36,36,14	74	2.99
L frontal gyrus, inferior	-38,36,14	50	3.10
HE group > FAS/PFAS group			
Frontal			
R frontal gyrus, superior	6,56,28	81	1.85
R frontal gyrus, medial	8,48,32		2.17

L frontal gyrus, superior	-12,48,28		2.21	
R frontal gyurs, middle	40,36,18	186	3.51	
R sub-gyral	40,40,10		2.08	
L frontal gyrus, inferior	-34,34,16	48	2.93	
L frontal gyrus, middle	-40,42,24		2.33	
Temporal				
L fusiform gyrus	-38,-36,-22	64	2.08	
L sub-gyral	-34,-34,4	20	2.68	
Occipital				
L cuneus	-20,-82,6	22	2.31	

Note. Submaxima within clusters are shown under maxima in italics.

The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

Table 6.8.

Between group comparison of regions of interest network showing greater activation for Neutral faces compared to distorted images after cluster size correction (threshold p<.05; cluster sizes of 8.2 – 199.3 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group	No significant differences		
Control group > HE group			
Frontal			
R frontal gyrus, medial	8,28,-16	54	2.19
Temporal			
L fusiform gyrus	-48,-8,-30	41	2.51
Sub-lobar			
R caudate-head	6,12,-2	54	2.19
FAS/PFAS group > Control group			
Frontal			
L frontal gyrus, middle	-46,48,10	10319	4.92
R frontal gyrus, middle	38,56,-6		4.36
Temporal			
R fusiform gyrus	48,-6,-28	72	2.92
L sub-gyral	-34,-40,2	24	2.41
Occipital			
R fusiform gyrus	30,-56,-12	322	3.67
L cuneus	-16,-74,8	309	3.38
R cuneus	12,-82,2		3.27
L lingual gyrus	-18,-82,-22	321	3.57
L fusiform gyrus	-24,-70,-12		3.34
Sub-lobar			
R extra-nuclear	18,-14,20	1646	3.92
L extra-nuclear	-18,-22,22	26	3.97
FAS/PFAS group > HE group			
Frontal			
R frontal gyrus, superior	22,50,-4	282	2.63
R frontal gyrus, inferior	52,26,20	388	3.08
R frontal gyrus, middle	36,4,40		2.80
L frontal gyrus, middle	-40,52,-4	314	3.67
L frontal gyrus, superior	-34,58,0		2.74
Temporal			
R fusiform gyrus	50,-8,28	90	4.17
L fusiform gyrus	-42,-60,-16	125	2.85
Occipital			

L lingual gyrus	-14,-92,-6	177	3.10
R cuneus	14,-96,0		2.88
L fusiform gyrus	-22,-52,-12	125	2.64
Sub-lobar			
R extra-nuclear	18,-4,12	5598	3.64
extending to L frontal medial gyrus	-4,58,-4		3.55
L extra-nuclear	-16,-22,22	27	2.99
HE group > Control group			
Frontal			
R frontal gyrus, middle	48,38,18	283	3.21
R frontal gyrus, inferior	50,48,2		2.32
R frontal gyrus, medial	10,30,34	81	3.04
L frontal gyrus, middle	-46,46,10	591	3.91
L frontal gyrus, superior	-30,34,36		3.17
Temporal			
R fusiform gyrus	40,-42,-18	82	2.70
L sub-gyral	-34,-34,4	16	2.78
HE group > FAS/PFAS group	No significant differences		

Note.
Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

Contrasts of negative versus positive emotions. The behavioural data from the affective appraisal task from Study II (see *Chapter Five*, pp. 115-118) showed exposure group differences within specific negative emotions, in particular for angry faces. To further explore the fMRI data, the activations of the negative emotions (angry, sad, fear) were, therefore, examined individually. Although the distorted image controls well for perceptual, procedural, and motivational aspects of the task, the between-emotions contrast can provide more information regarding differences in the processing of different emotions.

Previous research has consistently shown that faces displaying a positive emotion are recognised significantly faster than faces showing a negative emotion (Alves, Aznar-Casanova & Fukusima, 2008; Crews & Harrison, 1994; Leppänen & Hietanen, 2004). Moreover, because the data from the current and other studies (Esteves & Öhman, 1993; Hughdal, Iverson, & Johnson,

1993) show that the neutral face is more difficult to appraise than the positive face and requires greater recruitment of neural regions to process, for this analysis each negative emotion was contrasted separately against the positive condition (happy faces). Positive affect is normative and easier to interpret as it involves a simpler, more straight-forward emotional display involving less facial muscle involvement (Hess et al., 1997). For current analyses, the conditions were contrasted in both directions, i.e., where regional activation for the angry, fear and sad condition is greater than for the positive condition and where regional activation for the positive condition is greater than for the angry, fear and sad condition.

Contrasting the three emotions separately against the positive condition showed a similar pattern of activation for each individual negative emotion (Table 6.9 - 6.11). For both contrasts 'angry greater than positive' (Table 6.9) and 'sad greater than positive' (Table 6.10), the control group showed a greater activation for many regions in comparison to the FAS/PFAS and HE groups, with similar differences in both diagnostic groups. In contrast, both the HE and FAS/PFAS showed no greater activations than the control group. The HE group, however, showed greater activation on some regions compared to the FAS/PFAS group. Hence, unlike for other contrasts, e.g., neutral against distorted, the control group activates the most regions.

Specifically, for angry faces, the controls showed greater activations in the left inferior frontal gyrus, left fusiform gyrus and the right cuneus compared to both the FAS/PFAS and HE groups. Additional areas for which controls showed greater activations than the FAS/PFAS group were, amongst others, the left middle temporal gyrus, right fusiform gyrus and the left parahippocampal gyrus extending into the amygdala. Further differences in activation between the controls and HE group were, amongst others, in the left and right precentral gyrus and right sublobar regions including the uncus and caudate head.

For sad faces, the controls showed greater activations in the right inferior, middle and superior frontal gyrus, left middle frontal gyrus, right lingual gyrus and left cuneus than both the FAS/PFAS and HE groups. Additional areas that showed greater activations for controls than the FAS/PFAS group were in the right fusiform gyrus and in subcortical regions such as the left and right caudate-body and left caudate-tail. Further differences in activation between the controls and HE group were found in the left fusiform gyrus, left anterior cingulate and the bilaterally in the putamen.

Similarly, when contrasting fearful faces with the positive stimuli (Table 6.11), the control group showed greater activation in numerous regions in comparison to both the FAS/PFAS and HE groups. The controls showed greater activations in the left superior, inferior and middle frontal gyrus, left precentral gyrus, and the left and right fusiform gyrus. Additional areas that showed greater activations between controls and the FAS/PFAS group were subcortical regions, such as the right caudate tail and right putamen. Differences in activation between the controls and HE group were found in the left caudate, left putamen and right anterior cingulate. Moreover, the FAS/PFAS did not show greater activation in any regions compared control group, nor did the HE group show greater activation in any regions compared to both the control and FAS/PFAS groups. However, the FAS/PFAS group showed greater activation in some regions including the left and right fusiform gyrus and right lentiform nucleus compared to the HE group.

To summarize, a comparison of the contrasts between the three negative emotions separately versus the positive emotions and the contrasts of the three negative emotions grouped together versus the positive, the results were very similar. For example, the number of regions activated was similar for group comparisons where Controls > FAS/PFAS, FAS/PFAS > Controls

and FAS/PFAS > HE. The main difference was for the comparison where HE > FAS/PFAS; when grouping the negative emotions together, there were a large number of regional activation differences but when compared separately, there were few to no differences.

Table 6.9.

Between group comparison of regions of interest network showing greater activation for angry faces compared to happy faces after cluster size correction (threshold p<.05; cluster sizes of 7.8 -156.2 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group			
Frontal			
R frontal gyrus, middle	34,20,36	4258	4.30
L frontal gyrus, middle	-40,14,40		3.83
L frontal gyrus, inferior	-52,30,18	337	3.69
Temporal			
R fusiform gyrus	50,-46,-18	455	3.06
R temporal gyrus, inferior	52,-56,-16		2.67
R temporal gyrus, middle	52,0,-28	80	1.80
L fusiform gyrus	-58,-14,-28	131	3.02
L temporal gyrus, middle	-44,0,-30		2.92
Occipital			
R cuneus	16,-86,10	150	2.63
R lingual gyrus	16,-88,-2		2.61
R fusiform gyrus	28,-56,-14	455	3.51
L fusiform gyrus	-24,-66,-16	439	2.96
Limbic			
L parahippocampal gyrus	-30,2,-20	67	3.36
L amygdala	-18,-4,-24		2.28
Sub-lobar			
L extra-nuclear	-12,-2,8	2897	3.94
R extra-nuclear	26,18,2		3.85
Control group > HE group			
Frontal			
R frontal gyrus, inferior	54,14,28	370	3.64
R precentral gyrus	60,0,26		3.18
R frontal gyrus, superior	12,52,28	837	2.90
L frontal gyrus, medial	-6,52,42		3.54
L frontal gyrus, superior	-16,54,28		3.46

L precentral gyrus	-50,2,40	701	4.10
L frontal gyrus, inferior	-46,2,24		3.77
L frontal gyrus, middle	-42,14,38		3.50
Temporal			
L sub-gyral	-36,-30,-8	25	4.05
Occipital			
R cuneus	20,-78,10	35	3.15
L fusiform gyrus	-20,-64,-16	129	2.54
Sub-lobar			
R extra-nuclear	24,-6,-10	1028	3.83
R uncus, limbic lobe	28,0,-26		3.33
R caudate head	8,16,-2		3.28
FAS/PFAS group > Control group	No significant differences		
FAS/PFAS group > HE group			
Frontal			
L frontal gyrus, medial	-6,52,42	29	2.40
Sub-lobar			
L extra-nuclear	-26,-36,10	16	2.71
HE group > Control group	No significant differences		
HE group > FAS/PFAS group			
Frontal			
R frontal gyrus, middle	34,20,38	148	3.10
R frontal gyrus, inferior	48,10,38		2.11
L frontal gyrus, medial	-8,50,12	96	2.49
L frontal gyrus, middle	-32,46,28	68	2.59
Occipital			
R lingual gyrus	16,-92,-6	40	2.74
R cuneus	6,-86,4	49	2.33
L cuneus	-2,-78,8		2.11
Limbic			
R anterior cingulate	4,50,2	96	1.74
Sub-lobar			
R extra-nuclear	20,10,14	69	3.32

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

Table 6.10.

Between group comparison of regions of interest network showing greater activation for sad faces compared to happy faces after cluster size correction (threshold p<.05; cluster sizes of 5.9 – 168.2 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group			
Frontal			
R frontal gyrus, inferior	54,44,0	68	2.57
R sub-gyral	36,48,4		2.27
R frontal gyrus, middle	34,22,34	444	3.86
extending to L declive of vermis	-24,-72,-16		2.88
R frontal gyrus, superior	34,64,-2	79	2.05
L frontal gyrus, middle	-44,50,6	134	2.87
L frontal gyrus, inferior	-44,42,0		2.80
Occipital			
R fusiform gyrus	24,-80,-18	54	3.31
R lingual gyrus	4,-88,0	112	2.80
L sub-gyral	-36,-56,-12	168	3.50
L cuneus	-18,-90,4	44	2.84
Sub-lobar			
R extra-nuclear	32,-24,-8	36	2.75
extending to R sub-gyral, temporal	38,-30,-8		2.64
R caudate-body	14,10,12	388	2.68
L caudate-body	-14,6,20	264	2.96
L caudate-tail	-34,-28,-8	39	2.49
extending to L sub-gyral, temporal	-36,-34,-2		2.40
Control group > HE group			
Frontal			
R frontal gyrus, inferior	62,12,26	143	3.43
R precentral gyrus	56,2,40		3.14
R sub-gyral	26,30,36	183	3.13
R frontal gyrus, superior	34,34,38		2.78
R frontal gyrus, middle	32,24,34		2.29
L frontal gyrus, middle	-46,46,16	478	4.53
L frontal gyrus, inferior	-44,42,0		3.17
Occipital			
R lingual gyrus	10,-88,-2	106	2.73
L cuneus	-10,-88,6		2.41
Temporal			
R sub-gyral	38,-32,-4	37	2.42
L fusiform gyrus	-44,-52,-24	60	2.56
Limbic			

L anterior cingulate	-2,14,28	436	3.42
Sub-lobar			
R extra-nuclear	30,-10,12	473	3.40
R putamen	22,-2,4		2.93
L putamen	-26,6,8	138	2.60
L extra-nuclear	-30,0,2		2.43
FAS/PFAS group > Control group	No significant differences		
FAS/PFAS group > HE group			
Frontal			
L frontal gyrus, inferior	-42,44,12	69	2.86
L anterior cingulate	-2,14,28	59	2.54
HE group > Control group	No significant differences		
HE Group > FAS/PFAS Group			
Frontal			
R frontal gyrus, middle	48,12,34	232	2.90
L sub-gyral	-14,44,4	212	2.62
extending to anterior cingulate, limbic	10,40,2		2.26
Occipital			
R fusiform gyrus	26,-78,-18	45	3.28

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

Table 6.11.

Between group comparison of regions of interest network showing greater activation for fearful faces compared to happy faces after cluster size correction (threshold p<.05; cluster sizes of 8.2 – 139.5 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group			
Frontal			
R frontal gyrus, middle	34,22,34	170	3.27
R sub-gyral	26,30,34		3.22
L frontal gyrus, middle	-26,64,12	226	2.45
L frontal gyrus, medial	-4,48,32	259	2.97
L frontal gyrus, superior	-14,50,38		2.51
L precentral gyrus	-46,-6,24	89	2.81
L frontal gyrus, inferior	-52,4,32		2.13
Occipital			
R fusiform gyrus	26,-56,-14	58	2.97
R cuneus	16,-86,8	68	2.53
L fusiform gyrus	-32,-50,-14	79	2.88
Temporal			
L fusiform gyrus	-60,-12,-28	72	3.16
Sub-lobar			
R caudate tail	36,-26,-8	33	2.60
R putamen	16,12,-10	495	2.55
L extra-nuclear	-16,8,22	272	2.93
Control group > HE group			
Frontal			
R frontal gyrus, inferior	60,10,28	908	3.35
R frontal gyrus, superior	26,32,38		3.33
L frontal gyrus, superior	-8,52,38	286	2.91
L frontal gyrus, inferior	-46,0,24	515	4.84
L precentral gyrus	-34,2,30		2.89
L frontal gyrus, middle	-46,46,16	459	3.50
Temporal			
L fusiform gyrus	-40,-42,-18	310	2.46
L caudate	-36,-28,-8	27	3.02
Occipital			
R fusiform gyrus	34,-66,-14	260	2.91
extending to R fusiform gyrus temporal	36,-56,-18		2.72
extending to R parahippocampal gyrus	26,-40,-16		2.28
Sub-lobar			

R anterior cingulate	10,14,28	1409	3.08
L caudate-head	-6,8,2	788	3.44
L putamen	-24,10,-4		2.67
FAS/PFAS group > Control group	No significant differences		
FAS/PFAS group > HE group			
Frontal			
R frontal gyrus, middle	24,56,-8	42	2.21
R frontal gyrus, superior	22,58,2		1.76
L frontal gyrus, middle	-44,48,8	36	2.19
Temporal			
R fusiform gyrus	38,-58,-18	66	2.15
L fusiform gyrus	-44,-62,-20	141	2.55
L temporal gyrus, inferior	-52,-62,-16		2.51
Lenitform nucleus			
R lateral globus pallidus	26,-12,-2	118	2.29
R putamen	30,-22,4		2.51
HE group > Control group	No significant differences		
HE group > FAS/PFAS group	No significant differences		

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

Additional analyses were conducted examining activation patterns by contrasting greater regional activations of positive (happy) stimuli against each negative emotion (see Appendices P, Q and R). In contrast to the comparison of Angry > Happy, the activations for Happy > Angry showed no greater activations in any regions for control participants compared to both the FAS/PFAS and HE groups. Whereas for the comparison Angry > Happy, three FAS/PFAS groups showed very few to no significant greater activation compared to the other the HE and control groups, for the comparison Happy > Angry, the FAS/PFAS group showed greater activation in many regions when compared to the other two groups. Specifically, the FAS/PFAS showed greater activation in the right and left middle frontal gyrus, left medial frontal gyrus, right cuneus, and in left and right extra-nuclear sub-lobar regions more greatly compared to both other groups. Additionally, the FAS/PFAS group showed greater activation in the right and left

fusiform gyrus in both temporal and occipital regions compared to the control group. Whereas for Angry > Happy, the HE group did not show greater activation compared to the control group, for Happy > Angry, the HE group show greater activation in an array of left and right frontal gyrus regions, the right cuneus, right uncus and left caudate head when compared to the control group. For Happy > Angry, only two regions were activated more when comparing the HE group to the FAS/PFAS group in contrast to an array of significant between-group activation differences for Angry > Happy.

In contrast to the comparison for Sad > Happy, in which several regional between-group differences were found between the controls and both other groups, when looking at the activations for Happy > Sad, the control group showed no greater activations in any regions compared to both the FAS/PFAS and HE groups. Similarly, for Sad > Happy, the FAS/PFAS showed no greater regional activation compared to controls, for Happy > Sad, the FAS/PFAS group showed greater activation in an array of regions compared to control participants. These regions included areas within the right (middle, inferior and superior) and left (inferior and middle) frontal gyrus, the left lingual gyrus, right fusiform gyrus, right parahippocampal gyrus, the left caudate body and tail and the right caudate body and head. For Happy > Sad, the FAS/PFAS group showed greater activation in the right middle frontal gyrus, right fusiform gyrus and right and left anterior cingulate compared to the HE group. Whereas for Sad > Happy, the HE group had shown no greater activations compared to the control group, for Happy > Sad multiple significant activation differences were shown. These regions included right (inferior, middle, precentral and superior) and left (middle and inferior) frontal gyrus regions, right lingual gyrus and the left fusiform gyrus. Only two regions (left inferior frontal gyrus and left anterior cingulate) showed greater activation in the HE group compared to the FAS/PFAS group.

In contrast to the comparison of Fear > Happy, where several regional between-group differences were shown between the controls and both other groups, when looking at the activations for Happy > Fear, the control group showed no greater activations in any regions compared to both the FAS/PFAS and HE groups. Similarly, where for Fear > Happy, the FAS/PFAS showed no greater regional activation compared to controls, for Happy > Fear, the FAS/PFAS group showed greater activation in an array of regions compared to control participants. These regions included areas within the right middle and left (inferior, medial, middle, precentral and superior) frontal gyrus, the left (temporal and occipital) and right fusiform gyrus, right cuneus and the right caudate tail and putamen. The FAS/PFAS group showed no greater activations compared to the HE group. Whereas for Fear > Happy, the HE group had shown no greater activations compared to both the FAS/PFAS and control groups, for Happy > Fear multiple significant activation differences were shown. These regions included right (middle and superior) and left (inferior, middle and precentral) frontal gyrus regions, right and left fusiform gyrus and the right putamen.

In summary, regarding the activation patterns when comparing the three negative emotions separately to happy, the following overall observation was made: when comparing Happy > Angry, Sad and Fear, control participants did not show greater activation in any regions compared to both the FAS/PFAS and HE groups; whereas for Angry, Sad and Fear > Happy, controls showed greater activation in numerous regions across all comparisons. Additionally, when comparing greater activations of both alcohol exposed groups to controls, when Happy > Angry, Sad and Fear, many regional activation differences are observed; whereas when Angry, Sad and Fear > Happy, the same group comparisons showed no significant activation differences. When the three negative emotions were grouped together, much like when contrasted

individually, control participants activated significantly more regions compared to both alcohol groups. Similarly, there were very few to no differences found for FAS/PFAS participants when the negative emotions were grouped together or compared separately. Therefore, for the remaining analyses the three negative emotions were grouped together as one negative emotion.

Controlling for potential confounders. To confirm that the group differences seen are truly due to differences in PAE, four demographic variables were examined as potential confounders: child sex and age at scan, and maternal education and smoking during pregnancy were entered as covariates during the second-level analysis. The three tables below show the regions with increased activation for positive faces (Table 6.12), negative faces (Table 6.13) and neutral faces (Table 6.14) compared with distorted images prior and after control for potential confounders.

Table 6.12 shows that for the positive face condition, activation differences between groups were only minimally affected after control of confounders. For the comparison in which FAS/PFAS group showed greater activations, all but two regions continued to be evident after confounders were controlled for. These regions were relatively small clusters, ranging from 17-45 voxels in size and were: the right lingual gyrus in comparison to the control group, and the right middle frontal gyrus in comparison to the HE group. For the comparison where HE > Controls, the right medial frontal gyrus and left superior frontal gyrus no longer showed greater activation after statistical adjustment for the demographic variables. Thus, activation patterns between the HE and Control groups, thus, remain similar after control of confounders. However, for the contrast HE > FAS/PFAS, after controlling for confounders, only the region with the previously largest cluster size, the right inferior frontal gyrus, still showed increased activation. In this comparison, all other frontal regions that previously showed a difference in activation were no longer significant.

Table 6.13 shows that for negative faces, the majority of activation differences persist when for the comparison FAS/PFAS > HE after controlling for demographic variables. Where prior to control of confounders, the control group showed greater activation in two regions (occipital and temporal) of the left fusiform gyrus in comparison to the FAS/PFAS group, only activation in the temporal area of the fusiform gyrus remained significant. Greater activation in the right putamen also loses significance after adjustment for confounders. Activation in all of the other regions remain significant. In the comparison for Control > HE, only activation in the right lingual gyrus loses significance after control of confounders. Thus, the majority of activation group differences remain after controlling for the four control variables.

As in the positive faces condition, in the negative faces condition the HE > FAS/PFAS group comparison was confounded by the demographic variables; all except two of the frontal regions (right middle frontal gyrus and left inferior frontal gyrus) were no longer significant after control for confounders. However, the difference in activation pertaining to the left fusiform gyrus and left cuneus persisted after adjusted for confounders. Activation patterns in which the HE > Controls were not altered by control for confounders.

Table 6.14 shows that for neutral faces, after the control of confounders, key regions no longer showed greater activation for FAS/PFAS > HE. Most significantly, where prior to adjusting for control variables, in comparison to the HE group the FAS/PFAS group showed greater activation in the occipital regions of the right fusiform gyrus and the occipital and temporal regions of the left fusiform gyrus, these differences in activation no longer existed after. For the comparison, HE > Controls, only a single region, the left superior frontal gyrus, no longer showed greater activation after control for confounders.

Table 6.12.

Regions showing greater activation for positive faces compared to distorted images (threshold p<.05) after controlling for four potential confounding variables simultaneously

Between group contrast	Regions activated prior to controlling for covariates	Regions activated after controlling for covariates
Control group > FAS/PFAS group	No significant differences	No significant differences
Control group > HE group	No significant differences	No significant differences
FAS/PFAS group > Control group	Frontal	
	Right frontal gyrus, inferior *Right frontal gyrus, middle* Left frontal gyrus, inferior Left frontal gyrus, middle	Right frontal gyrus, inferior *Right frontal gyrus, middle* Left frontal gyrus, inferior Left frontal gyrus, middle
	Occipital	
	Right lingual gyrus Right fusiform gyrus	No significant differences Right fusiform gyrus
	Temporal	
	Right sub-gyral *Right extra-nuclear*	No significant differences *Right extra-nuclear*
	Sub-lobar	
	Left caudate-head	Left caudate-head
FAS/PFAS group > HE group	Frontal	
	Right middle frontal gyrus	No significant differences
	Occipital	
	Right fusiform gyrus Right cuneus Right lingual gyrus	Right fusiform gyrus Right cuneus Right lingual gyrus
HE group > Control group	Frontal	
	Right frontal gyrus, superior *Right frontal gyrus, middle*	Right frontal gyrus, superior *Right frontal gyrus, middle*

	Right frontal gyrus, medial	No significant differences
	Right precentral gyrus	Right precentral gyrus
	Right frontal gyrus, inferior	*Right frontal gyrus, inferior*
	Left frontal gyrus, superior	No significant differences
	Left frontal gyrus, middle	Left frontal gyrus, middle
	Temporal	
	Left Sub-gyral	Left Sub-gyral
	Limbic	
	Left anterior cingulate	Left anterior cingulate
	Sub-lobar	
	Right extra-nuclear	Right extra-nuclear
	Left extra-nuclear	Left extra-nuclear
HE group > FAS/PFAS group	Frontal	
	Right frontal gyrus, inferior	Right frontal gyrus, inferior
	Right frontal gyrus, middle	No significant differences
	Right precentral gyrus	No significant differences
	Left frontal gyrus, inferior	No significant differences
	Left frontal gyrus, middle	No significant differences
	Sub-lobar	
	Right extra-nuclear	No significant differences

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.
A description of "no significant differences" in the right column indicates that after controlling for the potential confounders the region listed in the middle column no longer shows greater activation.

Table 6.13.

Regions showing greater activation for negative faces compared to distorted images (threshold p<.05) after controlling for four potential confounding variables simultaneously

Between group contrast	Regions activated prior to controlling for covariates	Regions activated after controlling for covariates
Control group > FAS/PFAS group	Frontal	
	Left frontal gyrus, medial	Left frontal gyrus, medial
	Temporal	
	Left fusiform gyrus	Left fusiform gyrus
	Occipital	
	Left fusiform gyrus	No significant differences
	Left cuneus	Left cuneus
	Limbic	
	Left anterior cingulate	Left anterior cingulate
	Sub-lobar	
	Right extra-nuclear	Right extra-nuclear
	Right putamen	No significant differences
Control group > HE group	Frontal	
	Right frontal gyrus, medial	R frontal gyrus, medial
	Occipital	
	Right cuneus	Right cuneus
	Right lingual gyrus	No significant differences
	Sub-lobar	
	Right caudate-head	Right caudate-head
FAS/PFAS group > Control group	No significant differences	No significant differences
FAS/PFAS group > HE group	Occipital	
	Right cuneus	Right cuneus
HE group > Control group	Frontal	
	Right frontal gyrus, superior	Right frontal gyrus, superior
	Right frontal gyrus, inferior	Right frontal gyrus, inferior
	Left frontal gyrus, inferior	Left frontal gyrus, inferior

HE group > FAS/PFAS group	Frontal	
	Right frontal gyrus, superior	No significant differences
	Right frontal gyrus, medial	No significant differences
	Left frontal gyrus, superior	No significant differences
	Right frontal gyrus, middle	Right frontal gyrus, middle
	Right sub-gyral	No significant differences
	Left frontal gyrus, inferior	Left frontal gyrus, inferior
	Left frontal gyrus, middle	No significant differences
	Temporal	
	Left fusiform gyrus	Left fusiform gyrus
	Left sub-gyral	Left sub-gyral
	Occipital	
	Left cuneus	Left cuneus

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right. A description of "no significant differences" in the right column indicates that after controlling for the potential confounders the region listed in the middle column no longer shows greater activation.

Table 6.14.

Regions showing greater activation for neutral faces compared to distorted images (threshold p<.05) after controlling for four potential confounding variables simultaneously

Between group contrast	Regions activated prior to controlling for covariates	Regions activated after controlling for covariates
Control group > FAS/PFAS group	No significant differences	No significant differences
Control group > HE group	Frontal	
	Right frontal gyrus, medial	Right frontal gyrus, medial
	Temporal	
	Left fusiform gyrus	Left fusiform gyrus
	Sub-lobar	
	Right caudate-head	Right caudate-head
FAS/PFAS group > Control group	Frontal	
	Right frontal gyrus, middle	Right frontal gyrus, middle
	Left frontal gyrus, middle	Left frontal gyrus, middle
	Occipital	
	Right fusiform gyrus	Right fusiform gyrus
	Right cuneus	Right cuneus
	Left fusiform gyrus	Left fusiform gyrus
	Left lingual gyrus	Left lingual gyrus
	Left cuneus	Left cuneus
	Temporal	
	Right fusiform gyrus	Right fusiform gyrus
	Left sub-gyral	Left sub-gyral
	Sub-lobar	
	Right extra-nuclear	Right extra-nuclear
	Left extra-nuclear	Left extra-nuclear
FAS/PFAS group > HE group	Frontal	
	Left frontal gyrus, middle	Left frontal gyrus, middle

	Left frontal gyrus, superior	Left frontal gyrus, superior
	Left frontal gyrus, medial	Left frontal gyrus, medial
	Right frontal gyrus, superior	Right frontal gyrus, superior
	Right frontal gyrus, inferior	Right frontal gyrus, inferior
	Right frontal gyrus, middle	Right frontal gyrus, middle
	Occipital	
	Left lingual gyrus	Left lingual gyrus
	Left fusiform gyrus	No significant differences
	Right cuneus	Right cuneus
	Right fusiform gyrus	No significant differences
	Temporal	
	Left fusiform gyrus	No significant differences
	Right fusiform gyrus	Right fusiform gyrus
	Sub-lobar	
	Right extra-nuclear	Right extra-nuclear
	Left extra-nuclear	Left extra-nuclear
HE group > Control group	Frontal	
	Left frontal gyrus, middle	Left frontal gyrus, middle
	Left frontal gyrus, superior	No significant differences
	Right frontal gyrus, middle	Right frontal gyrus, middle
	Right frontal gyrus, inferior	Right frontal gyrus, inferior
	Temporal	
	Left sub-gyral	Left sub-gyral
	Right fusiform gyrus	Right fusiform gyrus
HE group > FAS/PFAS group	No significant differences	No significant differences

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right. A description of "no significant differences" in the right column indicates that after controlling for the potential confounders the region listed in the middle column no longer shows greater activation.

Examining potential mediators. Mediation by IQ and EF on the effects of PAE on neuronal activation within the ROI network was investigated separately. The WISC-IQ measure was added as a covariate to the second level analysis; in a second run the four domains of the EF were entered as four covariates in a second analysis. The tables below show the regions with increased activation for positive faces (Table 6.15) and negative faces (Table 6.16) in contrast to distorted images, prior and after the control for IQ and EF.

Table 6.15 shows that neither IQ nor EF mediate the effect of alcohol for the group comparisons for positive faces between the two alcohol-exposed groups, in the analyses in which the FAS/PFAS group shows greater regional activation. Only two minor regions are affected in the FAS/PFAS > Controls comparisons. In this contrast, activation of the right lingual gyrus is no longer significant after control for IQ. Also, both IQ and EF both mediate the activation of the right extra-nuclear region, a region with little known theoretical significance. Thus, the principal regions, such as an array of frontal gyrus regions, the right fusiform gyrus and left caudate head, were not altered after control for IQ and EF. Figure 6.6 is an activation map showing greater activation in the right fusiform gyrus during affective appraisal for positive faces compared to distorted images in children from the FAS/PFAS group compared to controls, after controlling IQ.

Otherwise, very few other minor regions lost significance after considering the mediating effect of either IQ and/or EF. For HE > Controls, these regions were two minor sub-gyral regions (right and left) mediated by EF. Similarly, for the analyses in which the HE group showed greater activation compared to the FAS/PFAS group, IQ and EF both mediated a minor right extra-nuclear region and IQ also affected the activation of the right frontal inferior gyrus in this comparison. Overall, however, all activation differences in major regions with theoretical

significance were not lost after examining potential mediation of IQ and EF. These findings suggest that these differences in regional activation were direct effects of PAE on affective processing and not a consequence of the effects of exposure on cognition.

Table 6.16 shows that for negative faces, the activation patterns for which the FAS/PFAS group showed greater activation than both the HE and control groups and for which HE > Controls did not change after examining potential mediation of IQ and EF. For the comparison for Controls > FAS/PFAS, the left fusiform gyrus in the occipital regions is no longer significant when controlling for IQ. However, the activation in the temporal regions of the left fusiform gyrus persists. Again, overall, the principal areas activated in affective face processing remained significant for faces displaying negative emotions suggesting that observed between-group differences in activation were directly associated with the effect of PAE and not mediated by the effect of PAE on IQ or EF.

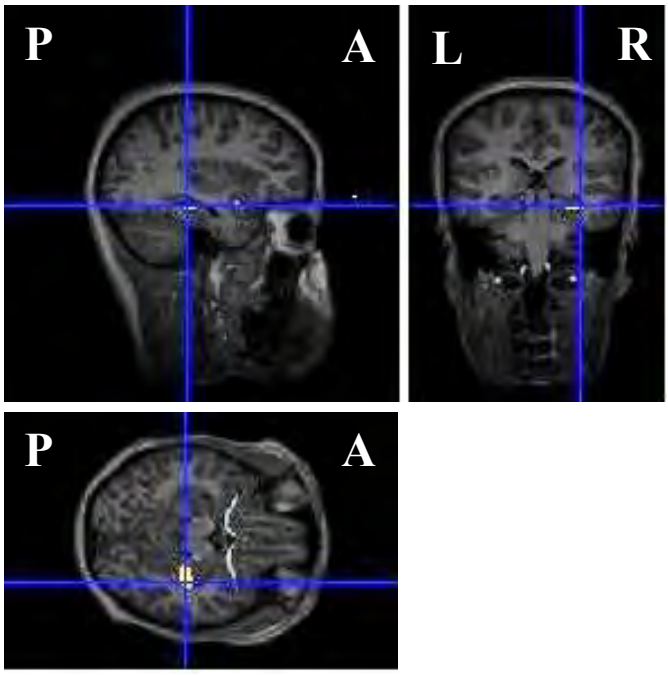

Figure 6.6. Right fusiform gyrus showing greater activation during affective appraisal for positive faces compared to distorted images in children from the FAS/PFAS group compared to controls after controlling IQ.

Table 6.15.

Cluster-size corrected regions showing greater activation for positive faces compared to distorted images prior and after controlling for IQ and EF (threshold p<.05)

Between group contrast	Regions activated prior to controlling for covariates	Regions activated after controlling for IQ	Regions activated after controlling for EF
Control group > FAS/PFAS group	No significant differences	No significant differences	No significant differences
Control group > HE group	No significant differences	No significant differences	No significant differences
FAS/PFAS group > Control group		Frontal	
	R frontal gyrus, inferior	R frontal gyrus, inferior	R frontal gyrus, inferior
	R frontal gyrus, middle	*R frontal gyrus, middle*	*R frontal gyrus, middle*
	L frontal gyrus, inferior	L frontal gyrus, inferior	L frontal gyrus, inferior
	L frontal gyrus, middle	L frontal gyrus, middle	L frontal gyrus, middle
		Occipital	
	R lingual gyrus	No significant differences	R lingual gyrus
	R fusiform gyrus	R fusiform gyrus	R fusiform gyrus
		Temporal	
	R sub-gyral	R sub-gyral	R sub-gyral
	R extra-nuclear	No significant differences	No significant differences
		Sub-lobar	
	L caudate-head	L caudate-head	L caudate-head
FAS/PFAS group > HE group		Frontal	
	R middle frontal gyrus	R middle frontal gyrus	R middle frontal gyrus
		Occipital	
	R fusiform gyrus	R fusiform gyrus	R fusiform gyrus
	R cuneus	R cuneus	R cuneus
	R lingual gyrus	R lingual gyrus	R lingual gyrus

HE group > Control group		Frontal	
	R frontal gyrus, superior	R frontal gyrus, superior	R frontal gyrus, superior
	R frontal gyrus, middle	*R frontal gyrus, middle*	*R frontal gyrus, middle*
	R frontal gyrus, medial	*R frontal gyrus, medial*	*R frontal gyrus, medial*
	R precentral gyrus	R precentral gyrus	R precentral gyrus
	R frontal gyrus, inferior	*R frontal gyrus, inferior*	*R frontal gyrus, inferior*
	R subgyral	R subgyral	No significant differences
	L frontal gyrus, superior	L frontal gyrus, superior	L frontal gyrus, superior
	L frontal gyrus, middle	L frontal gyrus, middle	L frontal gyrus, middle
		Temporal	
	L Sub-gyral	L Sub-gyral	No significant differences
		Limbic	
	L anterior cingulate	L anterior cingulate	L anterior cingulate
		Sub-lobar	
	R extra-nuclear	R extra-nuclear	R extra-nuclear
	L extra-nuclear	L extra-nuclear	L extra-nuclear
HE group > FAS/PFAS group	Frontal		
	R frontal gyrus, inferior	No significant differences	R frontal gyrus, inferior
	R frontal gyrus, middle	*R frontal gyrus, middle*	*R frontal gyrus, middle*
	R precentral gyrus	R precentral gyrus	R precentral gyrus
	L frontal gyrus, inferior	L frontal gyrus, inferior	L frontal gyrus, inferior
	L frontal gyrus, middle	*L frontal gyrus, middle*	*L frontal gyrus, middle*
		Sub-lobar	
	R extra-nuclear	No significant differences	No significant differences

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right. A description of "no significant differences" in the right column indicates that after controlling for the potential confounders the region listed in the middle column no longer shows greater activation.

Table 6.16.

Cluster-size corrected regions showing greater activation for negative faces compared to distorted images prior and after controlling for IQ and EF (threshold p<.05)

Between group contrast	Regions activated prior to controlling for covariates	Regions activated after controlling for IQ	Regions activated after controlling for EF
Control group > FAS/PFAS group		Frontal	
	L frontal gyrus, medial	L frontal gyrus, medial	L frontal gyrus, medial
	Temporal		
	L fusiform gyrus	L fusiform gyrus	L fusiform gyrus
		Occipital	
	L fusiform gyrus	No significant differences	L fusiform gyrus
	L cuneus	L cuneus	L cuneus
		Limbic	
	L anterior cingulate	L anterior cingulate	L anterior cingulate
		Sub-lobar	
	R extra-nuclear	R extra-nuclear	R extra-nuclear
	R putamen	No significant differences	No significant differences
Control group > HE group		Frontal	
	R frontal gyrus, medial	R frontal gyrus, medial	R frontal gyrus, medial
		Occipital	
	R cuneus	R cuneus	R cuneus
	R lingual gyrus	*R lingual gyrus*	no significant differences
		Sub-lobar	
	R caudate-head	R caudate-head	R caudate-head
FAS/PFAS group > Control group	No significant differences	No significant differences	No significant differences
FAS/PFAS group > HE group		Occipital	
	R cuneus	R cuneus	R cuneus
HE group > Control group		Frontal	
	R frontal gyrus, superior	R frontal gyrus, superior	R frontal gyrus, superior

HE group > FAS/PFAS group	R frontal gyrus, inferior L frontal gyrus, inferior	R frontal gyrus, inferior L frontal gyrus, inferior	R frontal gyrus, inferior L frontal gyrus, inferior
	Frontal		
	R frontal gyrus, superior *R frontal gyrus, medial* *L frontal gyrus, superior* R frontal gyrus, middle *R sub-gyral* L frontal gyrus, inferior *L frontal gyrus, middle*	R frontal gyrus, superior *R frontal gyrus, medial* *L frontal gyrus, superior* No significant differences No significant differences L frontal gyrus, inferior *L frontal gyrus, middle*	No significant differences *R frontal gyrus, medial* No significant differences R frontal gyrus, middle No significant differences L frontal gyrus, inferior *L frontal gyrus, middle*
	Temporal		
	L fusiform gyrus L sub-gyral	L fusiform gyrus L sub-gyral	L fusiform gyrus
	Occipital		
	L cuneus	L cuneus	L cuneus
	Sub-lobar		
	R extra-nuclear	R extra-nuclear	R extra-nuclear

Note. Submaxima within clusters are shown under maxima in italics.
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right. A description of "no significant differences" in the right column indicates that after controlling for the potential confounders the region listed in the middle column no longer shows greater activation.

Discussion

Neuroimaging studies have documented differences in the patterns of regional brain activation in FASD across numerous cognitive domains. This study is the first to examine patterns of regional brain activation in a task focusing on the affective domain. This chapter explores differences in neuronal activation during affective appraisal in relation to FASD diagnosis using fMRI. For this purpose, the regional activation of a neural affective appraisal network previously defined in the literature was examined across three different groups of children, aged 11-13 years, using an affective appraisal task in an MR scanner (Barbour et al., 2010; Diwadkar et al., 2012).

The affective appraisal task required the comparison of basic emotions of different valence displayed in a face and not the explicit identification of the emotion itself. The task was administered to children with FASD to explore potential differences in neuronal activation when completing a WM task with affective stimuli displaying positive, negative and neutral facial emotions. Performance accuracy on the affective appraisal task administered in the scanner did not differ between the FAS/PFAS, HE and control groups included in the fMRI analyses, confirming that the task that was administered was sufficiently simple and that all participant groups could complete it. However, the neuroimaging data show significant between-group differences in regional brain activations when completing the task. The behavioural data indicate that these differences in activation within the affective appraisal network investigated are not confounded by between-group differences in performance. The findings demonstrated here are consistent with the idea that the effect of PAE across diagnostic subgroups may differentially impair or alter neuronal functioning necessary for affective appraisal, an effect that varies depending on the valence of the affect displayed.

Overall, while there were group differences in activation, the initial whole-brain analysis showed no gross impairments across the three groups for the activation of brain regions when looking at faces in contrast to distorted control stimuli (non-face images). These findings confirmed that all participants processed faces as such.

The within-group ROI analyses, which looked at how the facial emotional network is activated, revealed that group differences in the underlying affective appraisal processes are indeed present. When all faces were contrasted against the distorted images, within-group ROI comparisons showed overlap of activation between the three groups. It is important that within each group the fusiform gyrus, which is involved in face recognition (Adolphs, 2002; Kanwisher, 2000; Kanwisher et al., 1997) showed greater activation when looking at faces in contrast to distorted (non-face) images. However, whereas the FAS/PFAS and control groups activated the fusiform gyrus bilaterally, the HE group showed activation unilaterally (right fusiform gyrus) only. This finding suggests an under-activation in the left fusiform gyrus when looking at face stimuli in non-syndromal children with heavy prenatal alcohol exposure. The reason for this pattern of group differences, which runs contrary to expectation, is unclear, and the finding warrants replication.

In the between-group ROI comparison, when all faces were contrasted to the distorted images, children with FAS/PFAS apparently needed to activate cortical regions more extensively to accomplish the same task than the control and HE groups. As expected, the between-group activation patterns show the greatest differences between the FAS/PFAS and control group. These activation differences include regions, such as the right and left fusiform gyrus, frontal gyri as well as some subcortical structures. Fewer activation differences were seen between the HE and control group. These findings were not related to the pattern of PAE since there were no significant differences in exposure levels, dose per

occasion, or frequency of drinking between the syndromal and non-syndromal alcohol-exposed groups.

When considering the affect displayed, it was shown that the type of facial emotion to be interpreted required greater activation in different cortical regions depending on their valence. For positive faces, the FAS/PFAS group showed greater activation compared to controls in major regions within the ROI networks, such as the right and left inferior and middle frontal gyri and the right fusiform gyrus. The FAS/PFAS group also showed greater activation in the right middle frontal gyrus, right fusiform gyrus, right lingual gyrus and right cuneus compared to the HE group. These differences were present even after control for IQ and EF, suggesting that differences in regional cortical activation are associated with the effect of PAE and not based on the effect of PAE on IQ or EF processes. The HE group showed greater activation in the right and left inferior and middle frontal gyri compared to the FAS/PFAS group and an array of frontal gyrus regions as well as the left anterior cingulate compared to controls. Therefore, for the positive emotion condition both the FAS/PFAS and HE groups appear to require greater activation in more cortical structures compared to controls. Moreover, positive faces appear to require greater processing of the static face stimuli in the FAS/PFAS group based on their greater activation of the right fusiform gyrus, compared to both the HE and control groups.

By contrast, for negative faces the FAS/PFAS group showed no greater activation compared to controls in regions associated with affective facial processing. Similarly, the HE group showed greater activation in only a few frontal gyrus regions. Instead, for the negative contrast, it was the control participants who showed greater cortical activation. These regions included the left fusiform gyrus, left anterior cingulate and left medial frontal gyrus compared to the FAS/PFAS group; and the right medial frontal gyrus, right cuneus and right caudate head compared to the HE group. Interestingly, for negative emotions, the HE group also

showed greater activation in the right (medial, middle & superior) and left (inferior, middle & superior) frontal gyrus, the left fusiform gyrus and left cuneus compared to the FAS/PFAS group. Thus, for negatively valenced emotions, the controls and HE groups appear to process affect more similarly to each other in contrast to the FAS/PFAS group. Their activation patterns suggest that they engage more with this more complex set of emotions compared to the easier positive condition, for reasons currently unknown. These regional cortical activation differences between syndromal and nonsyndromal children may contribute to understanding why HE participants show similar activation patterns to controls, despite the similar level and pattern of PAE between them and the FAS/PFAS group. Given the underactivation of the FAS/PFAS group when processing negative affective stimuli, together with the knowledge that these children do indeed demonstrate impairments in the social affective domain (Study I), one could hypothesize that PAE-related behavioural differences may likely emerge on a more complex affective appraisal task focusing on the discrimination of negative emotions.

Moreover, the reduced activation in the fusiform gyrus in individuals with FAS/PFAS suggests less extensive processing of the face itself. The differences shown in the left and right medial frontal regions, which persist after control for IQ and EF, may be part of a higher order affective appraisal processing network that functions independently of IQ and aspects of EF.

An examination in which the three negative emotions were analysed separately in contrast to the positive condition revealed a similar activation pattern for each of the negative emotions. Here, again the controls appear to engage the most with each negative emotion as they showed greater activation of regions (reviewed above) across all three negative emotions separately compared to both of the prenatal alcohol exposed groups. This finding confirms the previous pattern seen when contrasting the negative emotions together against distorted

images. It may suggest that because controls are able to recognise more easily that the negative emotions displayed are distinctly different from each other as well as from positive emotions, they engage with the stimuli more in order to appraise them and their unique characteristics correctly.

This pattern of activation can be explained by findings in previous literature looking at the effect of the valence of facial emotions. It is proposed that negative emotions are more heterogeneous and more difficult to express than positive emotions (Öhman, Lundqvist, & Esteves, 2001), which may explain why control participants, who have typical cognitive development, show more activation for negatively compared to positively displayed affect. Also, happy facial expressions are visually simpler than negative faces, as they can be recognized by a simple salient feature, such as a smile, and may thus not require an in-depth visual analysis (Adolphs et al., 2003).

Studies of recognition speed have shown that happy faces (positive valence) have a significantly lower recognition threshold than both neutral and negative faces (Esteves & Öhman, 1993). These findings suggest that a happy facial expression is easier to recognise and/or interpret than both a neutral and sad facial expression. Whereas it still unclear as to why this is the case, this may be because happy expressions are visually more distinctive compared to other emotional facial expressions (Leppänen & Hietanen, 2004). Happy expressions involve a greater change in the physical configuration of facial features compared to neutral faces, which are considered expressionless (Leppänen & Hietanen, 2004). This idea is supported by research that examined the relation between the physical intensity/degree of muscle activation in facial expressions and emotion recognition accuracy (Hess, Blairy, & Kleck, 1997). It was found that the accuracy rate for recognising negative emotions was related to the intensity of the facial expression. By contrast, happy faces had an extremely

high accuracy rate even at low intensity. Thus, one may infer that neutral and negative faces may be more similar to each other than neutral and positive faces.

Support for this point comes from research in which facial emotion recognition in adults with schizophrenia was compared to that of healthy controls. Patients with schizophrenia misidentified neutral facial expressions as negative (Kohler et al., 2003). This previous research may help explain why in the current study the control participants, who have typical affective processing, require less effort to interpret positive affect. Hence, the control participants appear to exhibit a valance-related activation pattern for both negative and positive affect consistent with that seen in previous literature and in contrast to the activation pattern found here in individuals with FAS/ PFAS.

These differences in processing different facial expression were also evident in the current study in which it was shown that neutral faces (compared to distorted/pixelated images) are more difficult to appraise than distorted faces and require more extensive neural activation to process. For example, the FAS/PFAS group activated a larger network of regions for the neutral versus distorted condition, in comparison to the HE and control group. Moreover, the FAS/PFAS also activated more cortical regions within the ROI especially in comparison to the HE group when processing neutral versus positive face stimuli. These additional regions included the right (superior and inferior) and left (middle and superior) frontal gyri, the left fusiform and lingual gyrus and sub-cortical areas. The interpretation of a neutral affect, thus, poses the greatest challenge for the FAS/PFAS group compared to negative or positive emotions. One may conclude that the degree of impairment and vulnerability in individuals diagnosed with FAS/PFAS results in the recruitment of a more extensive network to process the neutral affect. This is true in comparison to controls and the HE group.

Alternatively, the dysmorphic face, which is required for a diagnosis of FAS and PFAS, appears to provide a marker of severity of fetal alcohol effect. Both the control and non-syndromal HE groups appear to engage in a similar and less extensive neuronal network for the interpretation of the same neutral faces. This finding is consistent with previous literature, suggesting that the valence of neutral faces is generally more difficult to interpret than, for example, a happy face (Esteves & Öhman, 1993). Furthermore, studies on the development of facial emotion recognition have suggested that children's ability to recognise emotions emerges over time and varies depending on type of affect displayed (De Sonneville et al., 2002; Gross & Ballif, 1991; Smith & Walden, 1998; Vicari, Snitzer Reilly, Pasqualetti, Vizzotto, & Caltagirone., 2000). For example, happy facial emotions are recognised first and most accurately. The ability to recognise sadness, anger and then fear and surprise emerge later (Gross & Ballif, 1991).

Thus, one could argue that heavy PAE may impact the developmental trajectory of valence-related emotion recognition ability in children with FASD. However, whether the impairments are due to a delay or an arrest in development is unclear, yet, the fact that social-cognitive deficits appear to persist into adulthood, suggests the latter.

A vast number of neuroimaging studies in typically developing individuals have documented differences in regional activations related to the valence of the facial emotion expressed (see Fusar-Poli et al., 2009 for a Meta-analysis). Thus, valence-related between-group differences in activation are to be expected. However, the differences in cortical activation seen in the current study can be related to the effect of PAE; and valence-specific impairments in facial emotion processing have also been demonstrated in other clinical groups. For example, patients with clinical depression have a tendency to misrecognize happy faces as neutral and neutral faces as sad (e.g., Gur et al., 1992; Mandal & Bhattacharya, 1985). By contrast, patients with social anxiety disorder have been shown to

have difficulties in processing negative emotions including angry, disgust, fear and sad but not positive emotions such as happy and surprised (Montagne et al., 2006).

To assess this notion, that the observed differences in cortico-limbic activation are related to the effect of PAE on affective appraisal itself, rather than the effects of potential confounding variables or the effects of PAE on cognition, multiple regression analyses were run adjusting for confounders and to examine the mediating effect of IQ and EF. The four demographic control variables (child sex and age at scan, mother's age and maternal smoking during pregnancy) were adjusted in a single analysis, once for the positive emotion condition and once for the negative emotion condition. Overall, the analyses demonatrated that the control variables only minimally confounded the association between PAE and affective appraisal. The main observation was that after control of confounding variables, the activation patterns of the two alcohol exposed groups became more similar, yet some differences (reviewed above) persisted. However, the major differences in relation to the control group persisted, even after control for confounders.

The potential mediating impact of IQ and EF on the effect of PAE on the differences in cortical activation during affective appraisal was considered for both the positive and negative emotions. For the positive contrast, very few minor regions (reviewed in detail above) were not significant after control for both IQ and EF. Similarly, for the negative contrasts, very few regions were altered when IQ and EF were included in the analysis. Hence, the activations in which the groups differed changed only minimally, and the remaining major differences are independent of the association between PAE and overall cognitive competence, as indicated by IQ and EF. The one exception was for the comparison Controls > FAS/PFAS where the left fusiform gyrus in the occipital regions is no longer significant when controlling for IQ. Although the activation in the temporal regions of the left fusiform gyrus persists, this finding suggests that the general IQ deficit seen in children with

FASD may play a role in potential difficulties the FAS/PFAS group may have in the processing and recognition of faces.

Limitations and Future Directions

Neuroimaging research with individuals with FASD poses some unique challenges, which were considered in the methodological design of the current research. In a FASD population differences in cognitive ability are unavoidable as these are typical of the neurocognitive profile and a pre-requisite for an FASD diagnosis. In the current research I aimed at minimising the effect of cognitive ability and performance by including FASD and control participants who were recruited prospectively from the same community. Also, the affective appraisal task used here was chosen because it had been shown in Study II to elicit the similar behavioural performance between groups. Selecting a task that is less challenging and allows participant groups to perform at a similar level is a technique employed in neuroimaging research with individuals with FASD (e.g., Burden et al., 2009; Meintjes, et al., 2010; O'Hare et al., 2009) and other disorders.

Another limitation is that findings from this Cape Coloured population may not generalize to other populations. Given the prospective recruitment methods of this study (see the *Participants* Section, *Chapter Three*, p. 40), the current findings should be extendable to other South African, low SES communities with a high incidence of FASD. Moreover, the pattern of behavioural and growth findings for this cohort has been shown to be similar to performance in U.S. samples (e.g., Carter et al., 2013; Dodge et al., 2009; Lewis et al., 2015). It is, therefore, reasonable to assume that the neuroimaging findings would also be similar. Future studies should, nonetheless, examine these findings in other populations.

Another challenge in FASD-related neuroimaging research is that in most studies, participants are either older children or adolescents (Coles & Li, 2011). Given the developmental effects of PAE and age range used in this study, it is not clear whether the

observed deficits represent a developmental delay or reflect a permanent impairment. Thus, it is unclear whether the observed differences in activation may resolve over time or represent arrested development. A follow-up study administered at an older age or to an older sample should be conducted to determine whether the differences in neuronal activation during affective appraisal are permanent.

Common to other longitudinal studies investigating the effect of PAE, this study relies on the mother's report of prenatal alcohol consumption. However, at recruitment of the longitudinal cohort from which the current participants were recruited, alcohol consumption during pregnancy was determined prospectively by administering an alcohol ascertainment interview during the prenatal period. Also, the alcohol consumption protocol administered was validated in relation to report fatty acid ethyl esters in meconium samples in this community (Bearer et al., 2003) and also in relation to the alcohol-related cognitive impairments (e.g., Jacobson et al., 2002; 2004). As was done in Study I, future studies should, if possible, examine these neuroimaging outcomes in relation to continuous measures of alcohol exposure, which have been found to often be more sensitive in detecting adverse effects in FASD (e.g., DeGuio et al., 2014; Meintjes et al., 2015; Woods et al., 2015).

Conclusions

The face perception network includes visual regions, which are involved in the processing of static facial features, and limbic and prefrontal regions, which are used to evaluate the changeable features of a face. Thus, information extracted from face stimuli is processed across a network of cortical regions rather than a single ROI (Ishai, 2008). Hence, activation during face perception extends beyond the activation within the fusiform gyrus and is characterised by multiple activations throughout a network of structures. In typically developing individuals, the presentation of face stimuli elicits a bilateral activation of a specific cortical network (reviewed above) compared to the elicitation of scrambled faces

(Ishai, Schmidt, & Boesiger, 2005; Kranz & Ishai, 2006). The findings in the present study are consistent with this literature, demonstrating that processing of face stimuli extends beyond the activation of the fusiform gyrus. Moreover, between-group differences in activation were seen across the extensive face and facial affect processing network investigated in this study.

In summary, these data support the notion that heavy PAE alters activation within a cortical affective processing network, and differences in between-group activation patterns are related to the valence of the affective stimuli. These findings, thus provide additional evidence for a fetal alcohol-related deficit in social cognition due to impairments in affective processing.

For positive facial emotions, both alcohol exposed groups showed increased activation in more regions within the facial processing network in comparison to controls. This finding suggests that even for an emotion that is considered to be easier to interpret, as is the case with 'happy' (Leppänen & Hietanen, 2004), PAE groups require more effort to process this affect. In addition, the FAS/PFAS group also appear to over-activate right fusiform gyrus when processing happy facial affect, suggesting a possible impairment in the decoding of static face processing, a known function of the fusiform gyrus.

For the negative emotions, the FAS/PFAS group activated fewer regions compared to the control and HE groups. This was the case when comparing activation patterns for all three negative emotions (sad, angry, fear) grouped together, as well as separately. The finding that the FAS/PFAS group activate fewer regions for all three negative emotions suggests that, by contrast to the HE group, they devote less resources to distinguishing among negative emotions and may less readily distinguish the differences among them. It is possible that the HE and control groups activate more regions during the appraisal of the negative emotions because they are better able to recognise the three negative emotions as being distinctly

different and thus engage in greater detail in identifying and evaluating them, e.g., in categorising fear as fear and not simply as negative.

In the current study, analysis of the behavioural data suggested that children with PAE performed adequately on a relatively simple affective appraisal task. However, the analysis of the cortical activations during the task demonstrated that the neural processing of alcohol exposed children in this domain is not optimal compared to controls. This was shown in the neuronal activation patterns of children with FAS/PFAS where this group (1) required greater activation of regions within the network when interpreting faces displaying a happy affect, which is typically a simpler task than identifying negative emotions; and (2) recruited less extensive activations when processing negative facial emotions, which are more difficult to distinguish from each other. These fMRI data, thus, suggest that when individuals with PAE need to appraise affect in more challenging contexts, as would be the case, for example, in the course of dynamic social interaction, these individuals are likely to have difficulties in this domain. These findings are consistent with clinical reports that children with FASD frequently have difficulty "reading social cues" (Carmichael Olson et al., 1997; McGee et al., 2009; Streissguth et al., 1996). These data suggest that difficulty in affective appraisal contributes to the social interaction problems, for example, interpretation of facial social cues. Notably, in the current study, neither of the FASD groups was behaviourally impaired on the affective appraisal task, demonstrating that the differences in cortical activations were not confounded by deficits in the exposed groups' performance.

The current findings are consistent with other fMRI studies in the FASD literature, which suggest that a diagnosis of FAS may result in an overall decrease in the efficacy of neuronal functioning (Coles & Li, 2011). In this regard, the current study replicates previous findings which have demonstrated that depending on the nature of the task, this decreased efficiency may manifest itself in increased activation in task-specific cortical regions. An

fMRI study on number processing in children with FASD found that children with FAS recruited a more extensive range of cortical regions to perform the same task as control children after control for IQ and intracranial volume (Meintjes et al., 2010). Another fMRI study, which investigated verbal WM in children and adolescents with FASD, found increased activation in certain brain regions compared to control participants despite a lack of behavioural group differences (O'Hare et al., 2009). These effects persisted after control for IQ and the authors suggested that processing in these more extensive brain regions by children and adolescents with FASD is less efficient than in typically developing controls.

As hypothesised, it was confirmed that young adolescents with FASD exhibit differences in cortical activation on an affective appraisal task, despite the equivalent behavioural performance. These associations support the idea of alcohol-related effects of PAE on affective appraisal. Also, these effects appear to be independent of PAE's effect on overall cognitive ability and attention. Moreover, we showed that along the FASD spectrum there were differences in activation patterns for individuals with FAS/PFAS compared to both the HE and control children for the appraisal of faces independent of the valence they displayed. Here, differences in activation in the right fusiform gyrus may suggest that children with FAS/PFAS have a face perception problem, which may contribute to their inefficient processing of affective face stimuli.

Additionally, and more significant to the primary aims of the current study, the current data were consistent with the idea that an effect depending on the valence of the affect displayed also exists. Here, control participants demonstrate the typical activation pattern related to the valence of the affect displayed. For positively valenced emotions, the FAS/PFAS group, although showing greater activation in more regions, performed similarly to the HE group. Whereas for the negatively valenced emotions, the controls and HE groups

performed more similarly to each other in contrast to the FAS/PFAS group. We, therefore, confirm our second hypothesis.

The findings reviewed above are supportive of the idea that the between-group differences in cortical activation are specifically related to a social affective problem and not due to deficits in WM or secondary to other EF attentional problems; they are also not related to child age or sex. Instead, secondary behavioural problems (e.g., behavioural problems in school and trouble with the law; see e.g. Carmichael Olson et al., 1997; Streissguth et al., 1996) may be due in part to this primary social-cognitive deficit in affective processing at the neuronal level.

However, one argument from the current dissertation is that prior to interpreting the meaning and social relevance of facial emotions, which act as affective social cues determining our social interactions, facial emotions first need to be correctly recognized. Similarly, before facial emotions can be processed, the face displaying an affect needs to be processed. Thus, as much as facial emotion recognition can be viewed as a precursor to or the basis of facial emotion interpretation, facial processing can be viewed as a precursor to facial emotion recognition. Thus, given the current differences in activation in the fusiform gyrus, a structure primarily involved in the recognition of static facial features during facial perception, a general deficit in face perception, as a consequence of PAE, cannot be ruled out. Support for this point comes from findings in patients with schizophrenia, who have been shown to have a general deficit in face processing (Hooker & Park, 2002). Furthermore, deficits in facial affect recognition in the same patients was also related to social functioning, where a more accurate performance was significantly related to less dysfunction on specific domains of social functioning. Considering the possible stepwise exacerbation of impairments due to deficits in earlier and more basic facial social cue processing, further

exploration of the relation between facial affect recognition and face perception in children with FASD is warranted.

In conclusion, the general findings from the current study appear to have implications for understanding the social behavioural problems of children with FAS/PFAS and HE and can provide feedback for the design of remedial treatment paradigms. For example, if one considers the underactivation of the left fusiform gyrus by the FAS/PFAS group in comparison to controls for negative faces, yet, greater activation of the right fusiform gyrus for positive faces, it appears that interventions specifically desgined to examine faces rather than promoting more global attention intervention exercises *per se*, may improve the ability of affected individuals to interpret affective faces. Furthermore, exposed children not only activate more neuronal resources to process positive emotions but some, particularly those with FAS and PFAS, activate less extensively in discriminating the negative emotions. Thus, the emphasis for interventions should perhaps be placed on how to process affective stimuli more extensively, especially those displaying negative affect.

CHAPTER SEVEN: Dissertation discussion and synthesis

Overview

This dissertation examined social cognition in a cohort of South African children with a history of PAE and a diagnosis of FASD. The current chapter will review and summarize the dissertation research conducted across three studies, identify the main methods used and discuss their implications within the theoretical frameworks introduced. The novel findings and their contribution to the field will be reviewed within the context of future research directions.

FASD is the most widespread preventable form of mental retardation, and its severity is directly related to various individual maternal risk factors including maternal drinking patterns and genetic differences. The severity of impairment is generally related to the amount, timing and frequency of prenatal alcohol exposure. Aside from the severe cognitive impairments, social behavioural problems have long been recognized as a major debilitating consequence of PAE. Following anecdotal reports, research has in recent years increasingly focused on the teratogenic effect of PAE on social skills ability in children with FASD (see e.g., Kully-Martens et al., 2012; McGee et al., 2009; McGee et al., 2008; Rasmussen et al., 2013). Individuals with FAS have also been described as having difficulty understanding the consequences of behaviour, to be socially withdrawn, lacking knowledge about facial display rules and as being indifferent to subtle social cues (Streissguth, 1991). Thus, impairments in ToM ability including difficulties with affective processing, such as facial emotion recognition, may indeed be related to the poor social intelligence described in individuals with FASD.

The ability to identify emotions plays a critical role in effective social functioning as it aids the development of interpersonal and intergroup relationships and can limit social conflict (Fischer & Manstead, 2008). Therefore, inability to decode emotion could lead to

inappropriate behaviour and problems in social relations in everyday life (e.g., Ciarrochi, Chan, & Caputi, 2000; Marsh, Kozak, & Ambady, 2007).

In the current research project, the association of PAE and social cognition were investigated within two theoretical frameworks, namely Lemerise and Arsenio's (2000) modified SIP model and Halberstadt and colleagues model of ASC (2001; see *Chapter Two*, pg. 20-25). Both the SIP and ASC models attempt to break down the various processes and steps involved in social interaction. The models consider how social cues, such as facial emotional expressions, are encoded and interpreted and how a behavioural reaction, based on the processing of these social cues and personal previous experience, is then generated.

The present study was unique in its approach of examining the social deficits seen in children with FASD because it utilised two distinct yet overlapping cognitive concepts of social cognition, namely ToM and emotion recognition. ToM is referred to as the ability to infer and understand the mental states and beliefs of the people around us by, for example, interpreting another's facial expression. In order to attempt to interpret someone else's intent, one first needs to decipher the emotion or affect displayed in their face, and thus, recognizing other's emotions has been shown to be critical for ToM (Buitelaar & van der Wees, 1997; Phillips et al., 2002). A connection between ToM and emotion recognition is supported by neuroimaging studies showing similar patterns of brain activation when engaging in ToM and emotion recognition tasks (Campbell et al., 2006; Koshino et al., 2008; Stone et al., 2003).

Review of the Current Findings within the Theoretical Framework

The SIP model delineates the basic underlying cognitive processes involved in social interactions, which include face perception, affect recognition and the ability to access mental stores. These mental stores in turn help to explain the meaning of social cues and predict a possible consequence or provide a suitable reaction within the context of a situation. In the current research, the focus was to examine impairments in the processes outlined in steps one

and two of the SIP model, based on the notion that each step builds on the processing at the previous step. Thus, children with FASD have difficulties in later steps due to deficits in earlier steps. Therefore, the impact of a disruption in these early stage processes on (1) the remaining steps of the model and (2) future social-cognitive development was also considered. Investigation of social-cognitive impairment within the framework of the ASC model focused on the 'receiving' aspect of the model and its impact on the core and the processing of external social stimuli, i.e., those of others. A similar argument regarding the impact of impairments in affective processing on the inter-dependent processes in the SIP model can also be made for the ASC model. Namely, if children with FASD demonstrate difficulties in affect recognition they may also have deficits in identifying the meaning of affective messages and as a consequence develop inappropriate social schemas, which may lead to inappropriate behavioural outcomes.

In Study I of this dissertation, I employed a comprehensive developmentally sensitive ToM battery, as an initial exploration to determine whether deficits in specific aspects of ToM ability could be identified. I hypothesized that: (1) children with FASD would demonstrate deficits on ToM tasks in comparison to matched healthy controls. Moreover, children with FAS would perform worse on ToM tasks compared to children with PFAS who would perform worse than HE children who would perform worse than typically developing individuals; (2) children with FASD would demonstrate deficits on affect recognition tasks in comparison to matched healthy controls. Furthermore, children with FAS would perform worse on affect recognition tasks compared to children with PFAS who would perform worse than HE children who would perform worse than typically developing individuals; (3) the potential deficits in ToM and affect recognition in young adolescents with FASD would be present even when general cognitive and executive impairments are controlled for; and (4)

the association between PAE and ToM and affect recognition would be more sensitive when examined using a measure of continuous alcohol independent of FASD diagnosis.

The only significant group difference was on the RME task, which tests the ability to read other's mental states just by looking at the eye region of a face. The associations demonstrated in Study I supported the idea that PAE significantly impacts RME and appears to be independent of the effect of alcohol on general cognitive ability. There was a distinct pattern where children on the more severe end of the fetal alcohol spectrum, i.e., children with FAS and PFAS performed worse in comparison to heavily exposed non-syndromal children who in turn performed more poorly than typically developing non-exposed control children.

The modified SIP model incorporates the important and influential role emotion plays at every sequential step. The first step involves the encoding of affective cues of others and the recognition of the facial emotions, in which the eyes play a crucial role. The second step involves the interpretation of these cues by making causal attributions, judgments about intent and extensive evaluation based on past experience and the context of the situation. This type of interpretation of affective cues is part of the process in ToM. A central aspect of social information processing is the correct processing, including the perception and interpretation, of the emotions of others and is considered crucial to our social interactions (Kerns et al., 2016). The findings of Study I supported that idea that children with FASD have alcohol-related difficulties with attributing other's mental states based on the reading and interpretation of social cues communicated through people's facial eye region. Hence, these data suggest that the effect of PAE in the social information processing aspect of social cognition may lie embedded in the cognitive mechanisms comprising step two of the model. This impaired interpretation mechanism, which may lead to the incorrect deciphering of cues, has repercussions for the successive steps within the model. The model relies on the interplay

of an individual's personal experiences that shape the SIP network. The poor ability to correctly identify a person's mental state from their eye expressions, and thus misinterpreting the social context and making a misguided decision about the appropriate reaction, may be related to the social skills deficits seen in these children.

According to the model's hierarchical composition, interpreting social cues occurs early on in the social information process. While the model follows a sequential processing system its design also demonstrates how the core of the model, our internal database, continuously provides feedback, influencing the outcome and progression of each process at every step. The database is crucial to any future responses and decisions made during social information processing. Given the model's structure, one could infer that because individuals with FASD have deficits at the level of interpreting social cues, this impairment does not only affect the processes of the successive steps; but instead also directly impacts the acquisition of the knowledge store and social schemas forming the basis of our database. This in turn, could ultimately create a flawed database due to incorrect interpretations at step two, which may continue to impact processes at each level throughout the model, secondary to the specific alcohol-related effects rooted at step two. The findings of Study I, however, fail to inform whether perhaps the problem is at an earlier, more basic level of the SIP model, namely during the encoding of cues step. One proposition is that if the cues are already misrecognised and miscategorised at the first step of social information processing, the basis upon which the successive step, the interpretation of the cues, is constructed is the true issue.

Unlike the SIP model, the pin-wheel type construct of the ASC model does not follow a hierarchical progression. Instead, the ASC model comprises an integrated system with a continuous flow, which develops from the personal experience of receiving and sending affective messages. This basis of ASC in turn shapes the core of the ASC model, the self, which ultimately governs an individual's social interactions. Similarly, to the SIP model, the

ASC model incorporates awareness and interpretation of social cues. Within the affective social competence framework, the focus of the current research was not on the concepts of 'experiencing' and 'sending' affective messages for the development of social-cognitive abilities, but rather aimed at examining possible deficits within the 'receiving' branch of affective social competence. The 'receiving' component is an integrated part of affective social competence and includes the awareness of a message, identifying the meaning thereof and understanding the meaning within a set of display rules. These processes are in part governed by the input received specifically from the core of the model, the 'self', but also by all other interlinked processes embedded in the 'experiencing' and 'sending' components of the model. Both the awareness and the identification of affective messages are used in the recognition of affective facial expression. Additionally, the understanding of affective cues is also imperative to the flow of the ASC model. The findings of Study I suggest that children with FASD have difficulty in understanding the meaning and forming the correct interpretation of the affective facial cues they are presented with. The three main aspects of the ASC model are interlinked, and processes within these aspects are relayed through the core of the model. However, the core is also simultaneously constructed and shaped by a person's past experiences and interactions across all three components, the maintenance of appropriate concordance between them, as well as the context of past social situations.

Similarly, to the construct of the SIP model, where flaws in earlier steps will influence the development and processes in later steps, impairments within the receiving, sending and experiencing aspects of affective social competence, may impact the concordance between them. Furthermore, deficits in any of these processes may possibly create a flawed world view within the core including skewed knowledge of display rules, which in turn project and influence the processes throughout the model.

The link between 'receiving' and 'experiencing' appears to be particularly relevant to the context of the current research. The interplay between processing external stimuli of others and the experience thereof rely on accurately judging other's affective states and also using knowledge of other's affective states to identify one's own. Hence, deficits within 'receiving' and understanding other's affective cues will in turn also affect our personal affective experience, which ultimately affects the processes throughout the entire model, which are the basis of our social interactions.

The alcohol-related impairments in mentalizing ability following the presentation of facial social cues, as shown by the findings of Study I, suggest a deficit in the 'receiving' component of the ASC model. More specifically, this impairment in RME affects the concordance between 'receiving' and 'experiencing' affective content, which is in part maintained by accurately judging other's affective states. Due to the pin-wheel like structure, the basis upon which the 'experiencing' component is built will thus also be affected. This in turn will have repercussions for the maintenance of the concordance between the 'experiencing' and 'sending' component of the affective social competence framework, which ultimately determines the behavioural outcome.

Much like in the SIP model, Study I also fails to explain whether the deficits seen in the concordance between the 'receiving' and 'experiencing' component of the ASC model are perhaps rooted in a more basic, initial process instead. In this regard, deficits may perhaps emerge at the level of the awareness or identification of affective messages, namely in correctly recognizing affective facial expressions, rather than in the correct interpretation thereof.

Hence, in Study II, I wanted to explore social cognition within the models' frameworks at a more basic cognitive skills level, namely emotion recognition. Specifically, I wanted to look at the appraisal of affective facial expressions, which ultimately is the basis

for encoding the cues and a necessary step preceding all other processes in both the SIP and ASC models. Here, I hypothesized that: (1) children with FASD would perform more poorly on a WM task compared to healthy controls and difficulties would be associated to the task's complexity level. Moreover, children with FAS would perform more poorly than children with PFAS, who would perform worse than the HE children, who would perform worse than typically developing children; (2) children with FASD would demonstrate sufficient WM capacity to engage in an affective appraisal task and there would be group differences in affective appraisal ability across the four participant groups. Overall, no performance differences were found between groups for general affective processing on the simple affective appraisal task employed. However, an alcohol-specific group difference was found related to the valence of the affective stimuli, specifically for faces displaying angry affect. To establish potential differences in the recruitment of neuroanatomical structures and networks, the underlying cortical processes of affective appraisal were then further investigated.

Thus, the aim of Study III was to employ a behavioural and brain imaging paradigm that was related to previously observed alcohol-related RME deficits to determine whether the children were activating similar or different brain regions compared to controls and to identify atypical processing or potential compensatory mechanisms during affective appraisal. To my knowledge, Study III is the first to examine potential differences in the underlying neural network of affective appraisal in children with FASD. Here, I hypothesized that there would be a difference/variation in the degree to which: (1) FAS/PFAS, HE non-syndromal and control children recruit neural networks for affective appraisal; and (2) FAS/PFAS, HE non-syndromal and control children differ in the appraisal of positive, negative and neutral affect.

I confirmed that differences in the recruitment of neuroanatomical structures during affective appraisal related to heavy PAE do indeed exist. The atypical neuronal affective processing shown in children with FASD suggests that children with heavy PAE employ less efficient strategies to process the same affective social cues compared to their non-exposed peers. Furthermore, differences found in relation to the valence of the affective stimuli processed have demonstrated that individuals with PAE differentially process affective stimuli depending on the nature of the affective content, e.g., positive versus negative or neutral facial expressions.

The activation patterns reported in Study III raise the possibility that deficits in affective processing linked to social-cognitive impairments may extend beyond the abilities included in the models. Children with FAS/PFAS may have deficits in the general processing of faces. Face processing is a developmental ability (Bruce, et al., 2000; Cohen & Cashon, 2001; Donnelly & Hardin, 2003; Passarotti et al., 2003). Just as facial emotion recognition precedes the interpretation of emotions, general facial processing precedes the processing of facial emotions. Whereas difficulties in face processing and face recognition have been documented in other clinical populations, e.g., in children with autism (Chawarska & Volkmar, 2007; Dawson, Webb, & McPartland, 2005) and linked to abnormal neuronal activation patterns (Koshino et al., 2008), general face processing has not yet been examined in children with FASD. Given the findings from Study III, potential deficits in face processing, an ability that falls outside both the social information processing and affective social competence framework, need to be considered as a precursor to the impairments in receiving and encoding affective social cues, a process embedded in the first/basic levels of the models.

Current Findings and Previous Social Information Processing Research in FASD

Previous research applying the SIP model in FASD populations, looked at the model as a whole including all six steps (Mc Gee et al., 2009) or investigated the third and fourth step of the model (Stevens et al., 2012). McGee and colleagues assessed social information processsing by mimicking two types of social situation with the help of video vignettes, namely 'entering into a peer group' (Group Entry) and 'reaction to a provocative situation' (Provocation). Responses were recorded across various hypothetical scenarios assessing processes across all six steps of the SIP model. Results showed that children with a history of heavy PAE, aged 7-11 years, demonstrated difficulties on all six social information processing steps with different weaknesses depending on the type of the social situation. Specifically, in Group Entry scenarios, children with heavy PAE showed deficits in the social information processing processes of goal selection (step 3), response generation (step 4) and response evaluation (step 5) compared to non-exposed children. By contrast, for Provocation scenarios, compared to control children, children with PAE demonstrated difficulties in the processes of encoding (step 1), attribution (step 2), response evaluation (step 5), and enactment (step 6) within the SIP model. Consistent with these findings are the data of the current research, which found that the alcohol-related deficits in children with FASD are specific to the attribution of intent embedded in step 2 (Study I) and the underlying cortical processes during the identification of external emotions presented as affective social cues in the facial expressions of peers (Study III).

Stevens et al (2012) looked into the difficulties in social functioning seen in children with FASD by examining steps three and four of the SIP model. They investigated the social problem solving ability in children (mean age 12.6 years) by evaluating their responses to social dilemmas. Children with FASD produced fewer relevant responses, which suggested a reduced ability in generating solutions to social dilemmas. Although, steps three and four

were not directly assessed by the research of the current dissertation, the deficits found in the earlier steps may indeed impact on social problem solving ability, which may thus be a secondary effect of deficits in the more basic and earlier preceding steps of social information processing. Furthermore, impairments in any of the processing steps may also interact with known deficits in EF, which may further exacerbate and adversely affect social-cognitive ability in children with FASD.

Moreover, similarly to the current dissertation, one recent study (Kerns et al., 2016) focused on identifying deficits within the SIP model related to the perception and recognition of nonlanguage-based emotional cues, i.e. steps one and two. Kerns and colleagues examined the encoding of emotions displayed by others (step 1) and the decoding thereof (step 2) in children with FASD, aged 8-14, across different modalities: facial expressions, tone of voice, body positioning, and movement. Overall, they found that compared to age-matched healthy controls, children with FASD have greater difficulties in emotion recognition. However, the observed deficits may not be clinically significant. Also, these findings may be related to the age of the person displaying the affect, where children with FASD performed like control participants when the affect was displayed by a child, versus when the affect was displayed by an adult, where alcohol-exposed children performed significantly worse. Similarly, the current dissertation utilised the SIP model by first examining the processes embedded in step 2 (Study I) and then further investigating the basic mechanisms of step 1 (Study II & III). No previously published research was found that used ASC to explore social cognition in FASD.

In summary, as discussed above, the findings of the current research initially suggested an impairment in Step 2 of the SIP model and, similarly, the 'receiving' component of the ASC model based on alcohol-related differences on the RME. Furthermore, findings of Study III suggested that differences in neuronal activation may prevent the most efficient processing of affective stimuli at Step 1 of the SIP model and affecting processes of the

'receiving' aspect of affective social competence. Moreover, findings of Study III suggested that impairments following PAE may extend beyond the abilities included in both the models, and may arise due to deficits in face processing *per se*, a skill not incorporated in either of the models and preceeding facial affective processing.

Final Remarks

Limitations and Directions for Future Research

Since social cognition, and ToM in particular, follow a developmental trajectory the age range of participants recruited for Study I of the current research was specifically selected and limited to 9-11 years old (for an overview of the developmental trajectory of ToM see *Chapter Four*, p. 59). Therefore, the findings of the current research project may not be generalizable to other age groups, such as young adolescents or pre-school children. In Study I it was also suggested that ToM abilities that typically develop at a younger age, such as First- and Second-order False Belief, were intact. However, unless these basic ToM abilities are assessed around the age they typically develop, one cannot be certain whether these abilities are fully developed around the expected age or whether there is actually a delay in development, which is resolved by the time the child is between 9-11 years. Similarly, examination of RME at an older age would help to establish whether the ability of inferring mental states is simply developmentally delayed or permanently impaired. If the deficits indicate delayed development rather than permanent impairment, interventions could be designed to promote the development of the underdeveloped skill set. In the case of permanent impairment, however, intervention paradigms would need to target the employment and training of compensatory strategies to substitute the missing ability and the secondary behavioural problems that arise as a consequence.

Previous research in FASD has found that affected individuals have difficulties in the visual-spatial domain (Carmichael-Olson, Feldman et al., 1998; Mattson, Schoenfeld, &

Riley, 2001), which could affect configural face processing ability. Deficits in face processing could possibly result in difficulties with facial emotion recognition. The fusiform gyrus, which is part of the distributed cortical network elicited during face perception, was shown to have different activation patterns related to PAE as shown in Study III. This suggests that facial processing at the either the configural (whole face/configuration of features) or featural (individual facial features) processing level or both, may be the catalyst for difficulties in emotion recognition abilities, which in turn may be responsible for the social interaction deficits as reviewed above. Hence, an additional limitation of the current study is the failure to include and implement a control task to assess the effect of PAE on face processing ability. Establishing face processing ability in this cohort would have helped to clarify whether the impairment in affective appraisal is secondary to the effect of PAE on face processing or a direct consequence of exposure.

However, given the artificial and simplified measurement of affective appraisal employed in the current research, it can only be assumed that the atypical neuronal processing of affective cues becomes exacerbated in a more complex and naturalistic setting and may thus contribute to the social-cognitive deficits seen in individuals with FASD. Hence, future research should be designed to utilise more direct observation and examination of abilities within a more natural and realistic setting. Yet, interventions can possibly already be built on the current findings by, for example, devising behavioural training focusing specifically on the recognition, interpretation and social consequences of negatively-valenced affect.

Moreover, future research should also aim to establish the impact of an individual's own affective experience and display of facial emotions on the interlinked processes within SIP and ASC. Questions that need to be addressed include whether (1) deficits in the experience and sending of affective messages exist, (2) whether these exist as consequence of

the impairments in reading other's emotions and its impact on the interlinked processes, or (3) exist as a direct independent consequence of the effect of alcohol, or (4) as a combination of both.

Conclusion

In spite of these limitations, this is the first study to comprehensively assess ToM ability in children with FASD using a developmentally sensitive battery. Furthermore, this is the first study to assess affective appraisal in consideration of WM ability behaviourally and then using neuroimaging in an attempt to explain social-cognitive deficits by characterising atypical affective processing at the neuronal level. The robust methodologies employed in the current research project contributed to its strength and novelty. The prospective recruitment of participants makes this one of few research studies in the field of PAE that has detailed continuous data on prenatal alcohol consumption. These data could then be used to examine the impact of different patterns of exposure independent of FASD diagnosis. Furthermore, due to the location of the research setting, the unique binge-like maternal drinking pattern results in a good distribution of subtypes across the fetal alcohol spectrum, with an unusually high incidence of FAS. This allows for categorical group comparisons to be made between the different subtypes of FASD instead of treating the exposed individuals as a group without differentiating; or in the absence of a formal FASD diagnosis, simply grouped as individuals with a history of PAE. Also, typically developing children were recruited concurrently from the same community minimizing the impact in differences in external factors.

To summarise, in the current research project I investigated social cognition in children with FASD by exploring their abilities and impairments within the domain of social information processing and affective social competence. This aim was achieved by investigating (1) Theory of Mind and (2) emotion recognition ability, behaviourally and by means of neuroimaging, as potential determinants of deficits in social cognition, as these are

considered fundamental concepts necessary for successful social interaction. The findings of Study I showed that children with FAS and PFAS performed more poorly on the RME. This effect remained significant after control for IQ and EF, which I interpreted as an alcohol-related deficit in inferring people's mental states.

In Study II, a more basic level of social cue processing was examined by assessing the ability to read people's facial expressions because I considered potential impairments in this ability as a possible precursor to the deficits seen Study I. In addition, WM capability was assessed with two complexity levels (1-back and 2-back) as a potential confounder to the affective appraisal task administered. Here, I was able to demonstrate that all participant groups performed well on the 1-back, indicating ability to meet WM demands of the affective appraisal task but that an alcohol-related effect exists on the more complex 2-back WM task. On the affective appraisal task, no behavioural group differences were found, which confirmed the tasks suitability to possibly identify differences in neuronal activation, which is what I investigated in Study III by means of fMRI.

Although alcohol-exposed children performed as well as controls on the simple affective appraisal tasks administered in the scanner and no between-group differences were found, the notion that PAE is related to differences in regional cortical activations was supported. Specifically, compared to controls, children with FAS/PFAS had greater cortical activation in a network of structures when processing positive but less activation when processing negative facial affect. Based on these findings, which used a relative simple affective appraisal task, I suggested that when heavily exposed children need to appraise affect in more challenging contexts, like in day-to-day social interactions, the inefficient neuronal processing of affective stimuli would be exacerbated and possibly result in the social-cognitive impairments seen in these children. Hence, the current research project

provided insight into possible mechanisms for impaired social functioning based on solid evidence and research paradigms, and its findings have the potential to inform the design of future social-cognitive intervention programs.

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Appendix A

Wayne State University Ethics Approval

WAYNE STATE UNIVERSITY

IRB Administration Office
87 East Canfield, Second Floor
Detroit, Michigan 48201
Phone: (313) 577-1628
FAX: (313) 993-7122
http://irb.wayne.edu

NOTICE OF EXPEDITED AMENDMENT APPROVAL

To: Sandra Jacobson
Psychiatry
University Square Office Plaza

From: Dr. Scott Millis _____
Chairperson, Behavioral Institutional Review Board (B3)

Date: May 25, 2012

RE: IRB #: 026708B3F
Protocol Title: Neural Bases of Eyeblink Conditioning in FASD
Funding Source: Sponsor: NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
Sponsor: NATIONAL INSTITUTES OF HEALTH
Protocol #: 0802005726

Expiration Date: March 14, 2013

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

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The above-referenced protocol amendment, as itemized below, was reviewed by the Chairperson/designee of the Wayne
State University Institutional Review Board (B3) and is APPROVED effective immediately.

- Protocol – Change in treatment which includes collecting the blood draw at 1-3 weeks instead of 6 weeks. The earlier blood draw provides a more accurate reflection of iron transport across the placenta during pregnancy. This change does not affect risks to participants.
- Consent Form (dated 05/21/2012) – Parental Permission/Research Informed Consent (English and Afrikaans Versions) updated to reflect protocol changes.

WAYNE STATE UNIVERSITY

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NOTICE OF FULL BOARD AMENDMENT APPROVAL

To: Sandra Jacobson
Psychiatry
Department of Psychiatry and B

From: Dr. Scott Millis or designee
Chairperson, Behavioral Institutional Review Board (B3)

Date: July 18, 2013

RE:

IRB #:	026708B3F
Protocol Title:	Neural Bases of Eyeblink Conditioning in FASD
Funding Source:	Sponsor: NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM Sponsor: NATIONAL INSTITUTES OF HEALTH
Protocol #:	0802005726
Expiration Date:	February 20, 2014
Risk Level / Category:	45 CFR 46.404 - Research not involving greater than minimal riskResearch not involving greater than minimal risk

The above-referenced protocol amendment, as itemized below, was reviewed by the Wayne State University Institutional Review Board (B3) and is **APPROVED** effective immediately.

- Protocol – Change in enrollment criteria includes the addition of children ages 13-14 to complete the 2r phase of the longitudinal study. This change does not affect risks to participants.
- Oral Assent Script – Resubmission of Oral Assent Script for Ages 7-12 (English Version and Afrikaans Version).
- Assent Form (dated 6/4/2013) – Addition of Documentation of Adolescent Assent Form for Ages 13-14 (English Version and Afrikaans Version).
- Consent Form (dated 4/18/2013, Protocol Version #2r) - Parental Permission/Research Informed Consent (English Version and Afrikaans Version) updated to reflect change in age range and telephone number.
- Consent Form (dated 4/18/2013, Protocol Version #2rr Alternate) - Parental Permission/Research Informed Consent (English Version and Afrikaans Version) updated to reflect change in telephone number.

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NOTICE OF EXPEDITED AMENDMENT APPROVAL

To: Sandra Jacobson
Psychiatry
Department of Psychiatry and B

From: Dr. Deborah Ellis or designee
Chairperson, Behavioral Institutional Review Board (B3)

Date: June 11, 2014

RE:

IRB #:	026708B3F
Protocol Title:	Neural Bases of Eyeblink Conditioning in FASD
Funding Source:	Sponsor: NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM Sponsor: NATIONAL INSTITUTES OF HEALTH
Protocol #:	0802005726
Expiration Date:	February 19, 2015
Risk Level / Category:	45 CFR 46.404 - Research not involving greater than minimal riskResearch not involving greater than minimal risk

The above-referenced protocol amendment, as itemized below, was reviewed by the Chairperson/designee of the Wayne State University Institutional Review Board (B3) and is APPROVED effective immediately.

- Protocol - Enrollment criteria modified to reflect change in participants to be seen between ages of 8 to 13 years to ages of 8 to 17 years.
- Protocol - Other - Compensation modified to reflect change to Rand/Dollar conversion update. The compensation remains R150 regardless of USD.
- Consent Form - Parental Permission/Research Informed Consent - English and Afrikaans versions (revision dated 5/27/2014) - Consent Form modified to reflect change in enrollment criteria (increased age range to 8-17 years of age) and compensation amount of R150 due to conversion update between Rand and USD.

Appendix B

University of Cape Town Ethics Approval

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

Annual Progress Report

REC REF Number	187/2008
Title	Neural Bases of Eyeblink Conditioning in FASD
Principal Investigator	A/Prof E M Meintjes

List of documentation

RESEARCH ETHICS COMMITTEE
2011 -05- 19
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

HREC office use only (FWA00001637; IRB00001938)			
☐ Approved ✓	This serves as notification of annual approval, including all documentation described above.		
☐ Not approved	See attached comments.		
Type of review	☐ Expedited ✓	☐ Full committee	
Expiry date	30 MAY 2012		
Signature Chairperson of the HREC	Signed by candidate	Date	20/5/11

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30/5/2013
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signed by candidate	Date Signed	9/10/12

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	October 3, 2012		
HREC REF Number	187/2008	Current Ethics Approval was granted until	30/05/2012
Protocol title	Neural Bases of Eyeblink Conditioning in FASD		
Protocol number (if applicable)			
Principal Investigator	A/Prof EM Meintjes		
Department / Office Internal Mail Address	Department of Human Biology, Room 5.14 Anatomy Building, Faculty of Health Sciences, Anzio Road, Observatory		

1.1 Does this protocol receive US Federal funding?	X☐ Yes	☐ No
1.2 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	☐X No

2. List of documentation

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

UNIVERSITY OF CAPE TOWN

HUMAN RESEARCH ETHICS COMMITTEE
24 JUL 2013
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.5.2014
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signed by candidate	Date Signed	26/07/2013

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date form submitted	18 July 2013		
HREC REF Number	187/2008	Current Ethics Approval was granted until	30/5/2013
Protocol title	Neural Bases of Eyeblink Conditioning in FASD		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes X No		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	A/Prof EM Meintjes		
Department / Office Internal Mail Address	Department of Human Biology, Faculty of Health Sciences, University of Cape Town, Observatory, 7925		

1.1 Does this protocol receive US Federal funding?	X Yes	☐ No
1.2 Does this study require full committee approval?	☐ Yes	X No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Appendix C

English Consent form used for Studies I-III

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

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

Study Purpose: In this study we want to learn whether some aspects of a child's thinking and behavior are different when a mother drinks or and smokes during pregnancy, and whether genes (characteristics that you inherit from your parents) make it more or less likely that the child will show these differences. Other purposes of the study are to see whether your child's abilities when s/he was a baby and 5 years old predict how he or she is doing at 8-17 years of age. To help decide whether or not to agree to take part with your child in this study, a project staff member has talked with you about the risks and benefits of the study. This consent form summarizes the information given to you by the project staff member during this informed consent process.
The study will use new methods for studying the brain called MRI neuroimaging to better understand how drinking alcohol and smoking during pregnancy can affect a child's development. In neuroimaging, the child lies in a scanner that uses magnets to take pictures of the brain. In this part of the study, we will take pictures on the new scanner at Tygerberg Hospital while your child lies still and watches a video and does some simple finger tapping, attention, and memory tasks.

Study Procedures: If you agree to have your child take part in this study, we will bring you and your child to the our laboratory at the University of Cape Town (UCT) for 2-3 visits that will each take about 4 hours and to Tygerberg Hospital for 1-2 visits that should take about 3-4 hours in total.

During the visits to University of Cape Town, your child will do simple tasks involving finger tapping, attention, learning and memory, arithmetic, word meanings, puzzles, circle drawing, and mazes (Wechsler Intelligence Scale for Children; paced/unpaced finger tapping; Circle Drawing task; timing and pitch perception tasks; California Verbal Learning Test). We will test your child's vision.

In one task, your child will put on a special helmet. While your child is watching a video, a puff of air from the helmet will cause him/her to blink while hearing a tone. We will ask your child questions about the video afterwards.
We will weigh and measure your child and take a photograph to look for facial features that often relate to alcohol exposure during pregnancy.

During this visit, we will ask you some questions about your child's behavior and attention (Disruptive Behavior Disorders assessment), daily activities (Child Behavior Checklist), school and health history, and any medications that s/he is taking.
We will ask you to update us about stressful experiences in your daily life during the past year (Life Events Scale), your current drinking, smoking, and drug use, attention problems you may have had as a child (Barkley-Murphy ADHD Scale), and stressful feelings that you experience, including sadness, anxiety, and distress (Beck Depression Inventory; Structured Clinical Interview for DSM-IV).

At the end of the first visit, our research driver and nurse will take you and your child to a nearby clinic, where a technician/nurse will take a 5 cc blood sample (approximately 1 teaspoon) from your child's vein to test for lead and iron deficiency anemia. About 10 cc of blood (about 2 teaspoons) will be obtained from your child and yourself to study genetic differences that you and your child inherited from your family and have been found to be related to differences in alcohol use, depression, attachment, or child attention/behavior and development. These samples will be stored and used for future genetic analyses.
During the first visit to Tygerberg, your child will first practice the finger tapping, and attention and memory tasks s/he will be doing on a computer while lying in the scanner. During the neuroimaging, your child will lie on a padded plastic bed that slides into the scanner. We will ask him/her to lie as still as possible while the pictures are being taken. Taking these pictures of the brain does not hurt and is used every day by many people in the hospital. During the second visit to Tygerberg, our assistant will again practice the finger tapping and attention/memory tasks with your child and review with him/her the airpuff learning task that s/he has done in our laboratory at UCT. Your child will be shown special goggles that s/he will wear in the scanner and told that s/he will feel the airpuff and hear some tones while watching a video and that we will be asking him/her some questions about the video at the end of the scan. During some of the time in the scanner, your child will watch videos and during some of the time s/he will do the finger tapping and other tasks that were practiced before entering the scanner. There will be two sessions in the scanner at each visit to Tygerberg—both on the same day—one in the morning and one after lunch, which we will give you and your child while you are at Tygerberg. Each session in the scanner will last no longer than 45-60 minutes. Children with the following may not have an MRI but will take part in the rest of the visits: implanted medical devices, such as aneurysm clips in the brain, heart pacemakers, and cochlear (inner ear) implants; lead-based tattoos; or pieces of metal close to or in an important organ (such as, the eye); claustrophobia or fear of being in a small space.

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

Risks: None of the procedures we use at UCT or Tygerberg are dangerous for you or your child. The risks of drawing blood include some temporary discomfort or swelling, and rarely, infection. These risks that will be minimized because the procedure will be done by a trained phlebotomist (nurse/technician who has been specially trained to draw blood). We will begin by introducing you and your child to the research staff and will give you both breakfast each

day before the assessment begins. You will be present in a room nearby during all of your child's assessments and will be present with your child during the physical examination and blood draw. During the MRI neuroimaging assessments, certain metal objects, such as, watches, credit cards, hairpins, and writing pens, may be damaged by the MRI scanner or pulled away from the body by the magnet. For these reasons, we will ask your child to remove these before going into the scanner. When the scanner makes the pictures, the bed may shake, and your child will hear loud banging noises. S/he will be given earplugs or headphones to protect the ears. Also, some people feel nervous in a small closed space, such as when they are in the scanner. Your child will be able to see out of the scanner at all times, and we will not start until s/he tells us that s/he is comfortable. S/he will be able to stop the scanning at any time by squeezing a ball that s/he will hold in one hand and can talk to us using an intercom that is built into the scanner. There are no known harmful long-term effects of the magnetic fields used in this study. There is little risk that anything you tell us will be told to people outside the study and we will do everything we can to keep this information secret, as described below, except that evidence of child abuse or neglect will be reported to the appropriate authorities, as required by law, and may report other illegal activities that are reported to us during the visit.

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

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

Compensation: For taking part in this research study, we will give you R180 for each visit and a photo of your child, and we will give your child a small gift. You and your child will also be given breakfast and lunch each time you and your child come to University of Cape Town or Tygerberg Hospital.

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

Voluntary Participation/Withdrawal: Taking part in this study is voluntary. You may decide to have your child take part and later change your mind and quit the study. You and

your child are also free not to answer any questions or to stop any task before it is finished. Withdrawal from the study would not lead to any problems for you or your child. The researcher or the sponsor may also stop your child's taking part in this study without your agreeing to it.

Questions: If you have any questions now or in the future, you may contact Drs. Ernesta Meintjes or Christopher Molteno at 021-406-6291 or Dr. Sandra W. Jacobson at 001-313-993-5454. If you have questions or concerns about you or your child's rights as a research participant, you can contact the Chairs of either the University of Cape Town Research Ethics Committee (021 406-6338) or the Wayne State University Human Investigation Committee (001-313-577-1628).

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

_____	_____
Signature of Parent or Legally Authorized Guardian	Date
_____	_____
Printed Name of Parent or Authorized Guardian	Time
_____	_____
Oral Assent (children age 7-12 years)	Date
_____	_____
**Signature of Witness (When applicable)	Date
_____	_____
Printed Name of Witness	Time
_____	_____
Signature of Person Obtaining Consent	Date
_____	_____
Printed Name of Person Obtaining Consent	Time

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

Appendix D

Afrikaans Consent form used for Studies I-III

Toestemming deur Ouer/Ingeligte Toestemming tot Navorsing

Titel van Studie: Neurale Basis van Oogknip Kondisionering in FASD

Jy en u kind _____ word uitgenooi om deel te neem aan ons navorsingstudie. Lees asseblief hierdie vorm deur en vra vir ons enige vrae wat u het voordat u instem om in die studie te wees. Die mense wat hierdie studie doen is dokters en wetenskaplikes aan die Universiteit van Kaapstad se Fakulteit Gesondheidswetenskappe in Suid-Afrika en Wayne State Universiteit Mediese Skool in die Verenigde State: Ernesta Meintjes, Ph.D., en Christopher Molteno, M.D., van die Universiteit van Kaapstad, en Sandra W. Jacobson, PhD., en Joseph L. Jacobson, Ph.D., van Wayne State Universiteit in die Verenigde State. Die studie word geborg deur die Nasionale Instituut oor Alkohol Misbruik en Alkoholisme in die Verenigde State en die Departement van Wetenskap en Tegnologie en die Nasionale Navorsingsraad van Suid-Afrika.

Doel van die Studie: In hierdie studie wil ons leer hoe sommige aspekte van hoe ‘n kind dink en optree verskillend is wanneer ‘n ma drink en/of rook tydens swangerskap, en of gene (eienskappe wat jy van u ouers erf) dit meer of minder waarskynlik maak dat die kind hierdie verskille sal wys. Bykomende doelwitte van die studie is om te ondersoek die mate waartoe toetse wat gedoen is tydens die babajare en tydens 5-jarige ouderdom die kind se prestasie op 8-14-jarige ouderdom voorspel. Om u te help met u besluit om aan die studie deel te neem of nie, het ‘n projek personeellid die risiko’s en voordele met u bespreek. Hierdie toestemmingsvorm is ‘n opsomming van die inligting wat aan u gegee is deur die projek personeellid tydens hierdie ingligte toestemmingsproses.

Hierdie studie sal nuwe metodes wat MRI neurobeelding genoem word, gebruik om beter te verstaan hoe die drink van alkohol en rook tydens swangerskap ‘n kind se ontwikkeling kan affekteer. In neurobeelding lê die kind in ‘n skandeerder wat magnete gebruik om prentjies van die brein te neem. In hierdie deel van die studie sal ons prentjies neem met die nuwe skandeerder by Tygerberg Hospitaal terwyl u kind stil lê en na ‘n video kyk, en sekere eenvoudige take doen waartydens hy/sy sy/haar vingers moet tik, moet aandag gee, en sekere goed moet onthou.

Studie Prosedures: Indien jy instem om u kind aan hierdie studie te laat deelneem, sal ons u en u kind na ons laboratorium bring by die Universiteit van Kaapstad (UK) vir 2-3 besoeke wat elk ongeveer 4 ure sal duur, en na Tygerberg Hospitaal vir 1 - 2 besoeke wat elk omtrent 3-4 ure in totaal behoort te duur.

- Tydens die besoeke aan die Universiteit van Kaapstad sal u kind eenvoudige take doen waartydens hy/sy sy/haar vingers moet tik, moet aandag gee, dinge probeer onthou, somme doen, betekenis van woorde moet gee, legkaarte doen, doolhowe doen, en sirkels teken (Wechsler Intelligensie Skaal vir Kinders; vingertik taak; Sirkel Teken Taak, tyd en frekwensie persepsie take; Californieë Verbale Leer Toets).
- Ons sal u kind se visie toets / toets hoe goed u kind kan sien.
- In een taak sal u kind ‘n spesiale helm opsit. Terwyl u kind na ‘n video kyk, sal ‘n blasie lug uit die helm kom wat sal maak dat u kind sy/haar oog knip terwyl hy/sy ‘n geluid hoor.

- Ons sal u kind weeg en meet en ‘n foto neem om te kyk vir gesigskenmerke wat dikwels verbandhou met alkohol blootstelling tydens swangerskap.
- Tydens hierdie besoek sal ons u ook 'n paar vrae vra oor u kind se gedrag, vermoë om aandag te gee (Steurende Gedragsteuring Toets), daaglikse aktiwiteite (Kindergedrag Vraelys), skool en gesondheidsgeskiedenis, sowel as enige medikasie wat hy/sy neem.
- Ons sal u vra om ons op hoogte te bring oor stresvolle ervarings in u daaglikse lewe gedurende die afgelope jaar (Lewensgebeurtenis Skaal), u huidige drank- en dwelmgebruik en rookpatrone, probleme wat jy as ‘n kind mag gehad het om aandag te gee (Barkley-Murphy AAHV Skaal), en stresvolle gevoelens wat jy ervaar, insluitend hartseer, angs, en bekommernis (Beck Depressie Vraelys, Gestruktureerde Kliniese Onderhoud vir DSM-IV).
- Aan die einde van die eerste besoek sal ons navorsingsbestuurder en verpleegster u en u kind neem na 'n nabye kliniek, waar 'n tegnikus/verpleegster ‘n 5cc bloedmonster (ongeveer 1 teelepel) van u kind se aar sal neem om te toets vir lood en ystertekort anemie. Omtrent 10 cc bloed (ongeveer 2 teelepels) sal geneem word van u en u kind om genetiese verskille te bestudeer wat verband hou met verskille in alkohol metabolisme, depressie, gehegtheid, of die kind se aandag en ontwikkeling. Hierdie monsters sal gestoor word en gebruik word vir toekomstige genetiese analises.
- Tydens die eerste besoek aan Tygerberg, sal u kind eers die vingertik- en aandag en geheuetake oefen wat hy/sy op ‘n rekenaar sal doen terwyl hy/sy in die skandeerder lê. Gedurende die neurobeelding sal u kind op ‘n sagte plastiek bed lê wat in die skandeerder inskuif. Ons sal hom/haar vra om so stil as moontlik te lê terwyl die prentjies geneem word. Die afneem van hierdie prentjies (foto’s) van die brein maak nie seer nie en word elke dag deur baie mense in die hospitaal gebruik. Tydens die tweede besoek aan Tygerberg sal ons assistent weer die vingertik- en aandag/geheuetake met u kind oefen en met hom/haar hersien die lugblasie leertaak wat hy/sy in ons laboratorium by UK gedoen het. Tydens die skandeerbesoeke sal ons vir u kind spesiale brille wys wat hy/sy sal dra in die skandeerder. Ons sal vir u kind sê dat hy/sy die lugblasie sal voel en ‘n soort geluid sal hoor terwyl hy/sy na ‘n video kyk en dat ons vir hom/haar ‘n paar vrae oor die video sal vra aan die einde van die skandering. Vir 'n gedeelte van die tyd in die skandeerder sal u kind na videos kyk, en vir 'n gedeelte van die tyd sal hy of sy die vingertik en ander take doen wat ons geoefen het voordat hy/sy die skandeerder binnegegaan het. Daar sal gedurende elk van die besoeke aan Tygerberg twee sessies in die skandeerder wees – albei op dieselfde dag - een in die oggend en een na middagete. Ons sal vir u en u kind middagete gee terwyl julle by Tygerberg is. Elke sessie in die skandeerder sal niks langer as 45-60 minute duur nie. Kinders met enige van die volgende toestande mag nie 'n MRI onderneem nie: ingeplante mediese toestelle soos aneurisme knippies in die brein, hart pasaangeërs, en binne-oor inplantings; loodgebasseerde tattoeërmerke, of stukkies metaal naby aan of binne-in 'n belangrike orgaan (soos die oog); engtevrees of die vrees om binne 'n klein ruimte beperk te wees.

Voordele: Daar mag dalk geen direkte voordele vir u wees nie, maar inligting van hierdie studie mag ander mense help, nou of in die toekoms. Jy sal inligting ontvang oor u kind se huidige ontwikkeling op hierdie ouderdom. Ons sal die bevindings van hierdie studie slegs gebruik vir navorsingsdoeleindes. Indien 'n ernstige probleem egter gevind word, sal ons vir u sê en u kind verwys na 'n dokter en/of iemand wat kan help, indien jy dit wil hê. Indien u kind aan enige ernstige siekte ly, sal ons u na die Rooikruis Kinderhospitaal stuur. Geen inligting oor u kind sal uitgegee word aan enige dokters, hospitale, of skole tensy jy dit skriftelik versoek en toelaat nie.

Risiko's: Geen prosedures wat ons by UK of Tygerberg sal gebruik is gevaarlik vir u of u kind nie. Die risiko's van bloedtrek sluit soms 'n bietjie tydelike ongemak of swelling in, en by uitsondering, infeksie. Hierdie risiko's sal verminder word omdat die prosedure deur 'n opgeleide flebotomis (verpleegster/tegnikus wat spesiaal opgelei is om bloed te trek) gedoen sal word. Ons sal begin deur u en u kind aan die projekpersoneel bekend te stel en sal vir julle albei ontbyt gee elke dag voordat die toetse begin. Terwyl al u kind se toetse gedoen word sal jy in 'n vertrek naby u kind wees en jy sal saam met u kind wees tydens die fisiese ondersoek en wanneer die bloed getrek word. Tydens die MRI neurobeelding mag sekere voorwerpe soos horlosies, kredietkaarte, haarknippies en skryfpenne beskadig word deur die MRI skandeerder of deur die magnet weggetrek word van die liggaam. Om hierdie redes sal ons u kind vra om hierdie voorwerpe af te haal voordat hy/sy die skandeerder binnegaan. Wanneer die skandeerder die prentjies neem, mag die bed skud, en u kind sal harde kapgeluide hoor. Hy/sy sal oorpluisies en oorfone gegee word om sy/haar ore te beskerm. Sommige mense voel ook senuweeagtig in 'n klein beperkte spasie soos wanneer hulle in die skandeerder is. U kind sal te alle tye by die skandeerder kan uitsien, en ons sal nie begin voordat hy/sy nie vir ons sê dat hy/sy gemaklik is nie. Hy/sy sal ook enige tyd kan stop deur 'n bal te druk wat hy/sy in een hand sal vashou en hy/sy sal met ons kan praat deur 'n interkom wat in die skandeerder ingebou is. Sover almal weet is daar geen skadelike langtermyn effekte as gevolg van die magnetise velde wat in hierdie studie gebruik word nie. Daar is baie min kans dat enigiets wat jy vir ons vertel vir ander mense buite die studie gesê sal word en ons sal alles doen wat ons kan om hierdie inligting geheim te hou behalwe, soos hieronder beskryf, indien daar tekens is van kindermishandeling of –verwaarlosing sal dit egter aan die toepaslike owerhede gerapporteer word, soos deur die wet vereis. Ons mag ook ander onwettige aktiwiteite rapporteer wat aan ons tydens die besoek bekend gemaak word.

Navorsingsverwante Beserings: Indien jy of u kind tydens die studie beseer word sal jy behandeling ontvang wat insluit eerstehulp, noodbehandeling en opvolg-sorg soos benodig. Geen vergoeding, terugbetaling, of gratis mediese sorg word verskaf deur Wayne State Universiteit of die Universiteit van Kaapstad nie. Laat die navorser onmiddelik weet as jy dink dat u kind 'n navorsingsverwante besering opgedoen het.

Studiekostes: Daar sal geen koste wees vir u of u kind om aan hierdie navorsing deel te neem nie, en jy en u kind sal deur ons bestuurder vervoer word na die laboratorium by UK en Tygerberg Hospitaal.

Vergoeding: Vir u deelname aan hierdie navorsingstudie sal ons u R150 ($25) gee vir elke besoek en 'n foto van u kind, en vir u kind sal ons 'n klein geskenkie gee. Ons sal ook vir u en u kind ontbyt en middagete gee elke keer as julle na UK of Tygerberg Hospitaal toe kom.

Vertroulikheid: Ons sal alle inligting wat ons tydens die studie versamel oor u en u kind geheim hou tot die mate waartoe die wet dit toelaat. Hierdie inligting sal nie gebruik word op enige manier wat enigiemand anders sal toelaat om te weet wat jy of u kind vir ons vertel het nie, behalwe dat tekens van kindermishandeling of –verwaarlosing aan die toepaslike owerhede gerapporteer sal word, soos deur die wet vereis. Jy en u kind sal in ons navorsingsrekords slegs deur 'n kodenommer geïdentifiseer word en julle name sal nie op die rekords verskyn nie. Ons sal nie inligting uitgee wat u of u kind by name noem nie tensy jy ons skriftelik toestemming gee, maar u rekords mag hersien word deur die studie borg, die Menslike Navorsings Komitee by Wayne State Universiteit, of regeringsliggame met toepaslike regulatoriese oorsig. Die lys wat deelnemers se identifikasienommers met hul name verbind sal gestoor word in geslote kabinette in die navorsingslaboratorium. Slegs personeellede wat nodig het om u telefonies of persoonlik te kontak sal toegelaat word om na hierdie leêrs te kyk. Inligting vanaf hierdie studie, insluitend foto's en videos mag aangebied word by wetenskaplike vergaderings of

joernale of vir opleidingsdoeleindes gebruik word, maar u en u kind se name sal geheim gehou word.

Vrywillige Deelname/Onttrekking: Deelname aan hierdie studie is vrywillig. Jy mag besluit om u kind aan die studie te laat deelneem en later van besluit verander en die studie los. Jy en u kind is ook vry om enige vrae nie te beantwoord nie, of om enige taak te stop voordat dit klaar is. Onttrekking aan die studie sal geen probleme vir u of u kind veroorsaak nie. Die navorser of die borg mag u kind se deelname aan hierdie studie stop sonder dat jy daartoe instem.

Vrae: Indien jy enige vrae het nou of in die toekoms, kan jy Drs. Ernesta Meintjes of Christopher Molteno kontak by 021-406-6291 of Dr. Sandra W. Jacobson by 091-313-993-5454. Indien jy enige vrae of bekommernisse het oor u of u kind se regte as ‘n deelnemer aan die navorsing, kan jy die voorsitters kontak van die Universiteit van Kaapstad Navorsings-Etiek Komitee (021 406-6338) of die Wayne State Universiteit se Menslike Navorsings Komitees (001-313-577-1628).

Toestemming om aan ‘n Navorsingstudie deel te neem: Om vrywilliglik in te stem om u kind te laat deelneem aan hierdie studie, moet jy op die lyn hieronder teken. Indien jy besluit om met u kind deel te neem, mag jy of u kind enige tyd stop. Jy gee nie enige van u of u kind se regte op deur hierdie vorm te teken nie. U handtekening wys dat jy hierdie hele toestemmingsvorm gelees het of dat dit aan u voorgelees is, insluitend die risiko’s en voordele, en dat ons al u vrae beantwoord het. Ons sal vir u ‘n kopie van hierdie toestemmingsvorm gee om huis toe te neem.

_____ _____

Handtekening van Ouer of Wetlik Gemagtigde Voog Datum

_____ _____

Naam in drukskrif van Ouer of Wetlik Gemagtigde Voog Tyd

_____ _____

Mondelinge Instemming (kinders van ouderdom 7-12) Datum

_____ _____

**Handtekening van Getuie (wanneer van toepassing) Datum

_____ _____

Naam van Getuie in drukskrif Tyd

_____ _____

Handtekening van Persoon wat Toestemming neem Datum

_____ _____

Naam in drukskrif van Persoon wat Toestemming neem Tyd

**Gebruik wanneer toestemmingsvorm aan ouer voorgelees is (bv. wanneer ongeletterd, wetlik blind, vertaal in ‘n vreemde taal).

Appendix E

English Informed Assent Form used for Study III

Documentation of Adolescent Assent Form

(ages 13-17)

Title: Neural Bases of Eyeblink Conditioning in FASD

Study Investigator: Sandra W. Jacobson, Joseph L. Jacobson,

Christopher D. Molteno, Ernesta M. Meintjes

Why am I here?

This is a research study. Only people who choose to take part are included in research studies. You are being asked to take part in this study because you are one of a large group of children who have been taking part in this study since you were born and have taken part in visits as an infant and at 5 years of age. We are inviting you to take part in the next phase of this study. Please take time to make your decision. Talk to your family about it and be sure to ask questions about anything you don't understand.

Why are they doing this study?

This study is being done to find out how children learn and remember things and solve simple problems. We are trying to understand whether and how diet, alcohol, smoking, and drug exposure during pregnancy may affect development. We study children at different ages using different tasks to see how they grow and develop.

What will happen to me?

Here at University of Cape Town, we will be studying what happens when you feel a puff of air in your eye. You will sit in a chair wearing a special helmet and will watch a video. From time to time, you will feel a puff of air from the helmet and sometimes you will hear a tone. You will also do simple tasks involving tapping your finger, naming pictures, learning lists of words, reading and arithmetic, puzzles, mazes, memory and computer tasks, and tasks about how other people feel and understand another person's point of view. We will also weigh you, measure how tall you are, take a photo, and check how well you can see. You will spend this morning here and will come back to University of Cape Town another day to do the air puff task and the other tasks that I mentioned.

The second part of the study involves neuroimaging, which is a new way to learn about the brain by taking pictures of the brain. These pictures can help us better understand how the brain works. For this part of the study we will drive you and your mother to Tygerberg Hospital. During the neuroimaging, you will lie on a plastic bed that slides into a large machine called a scanner. We will ask you to lie as still as possible while the pictures are being taken. Taking these pictures of the brain does not hurt and is used everyday by many people in the hospital. During some of the time in the scanner, you will watch videos and during some of the time you will do simple tasks involving tapping your finger or doing simple puzzles, or reading and arithmetic, or learning and memory, or looking at pictures and figuring out if two people seem to have the same feeling. There will be one session in the scanner.

We will also ask you to give us a sample of your spit (saliva) and have a nurse take a small amount of blood from your arm to study how your genes (family characteristics that you get from your parents) affect how you do these tasks and how you act.

How long will I be in the study?

You will be in the study for this phase two days for about 3-4 hours at our laboratory at University of Cape Town (including breakfast, a snack, and lunch) and one visit involving about 45-50 minutes in the scanner and 1 hour of training and assessment outside the scanner at Tygerberg Hospital.

Will the study help me?

You will not benefit from being in this study; however, information from this study may help other people in the future better understand how the brain performs different tasks and whether diet, alcohol, smoking, or drug exposure during pregnancy affects how the brain performs.

While taking part in this phase of the research study, we will give you a small gift and a photo taken of your brain at the end of the scanning. We will provide breakfast, a snack, and lunch each time you come to our laboratory at University of Cape Town or Tygerberg Hospital.

Will anything bad happen to me?

There are no risks from being in the scanner at Tygerberg Hospital or from any of the tasks we do with you in our laboratory at University of Cape Town. The risk of drawing blood

include some temporary discomfort swelling and rarely infection. These risks will be small because the blood will be taken by a trained person (nurse/technician). Some people feel nervous in a small closed space, such as when they are in the scanner. You will practice what it is like in a pretend scanner beforehand. We will give you earplugs or headphones so that the loud banging of the scanner will not bother you. There is a button you can press to ask questions or stop the scan at anytime. You can see out of the scanner at all times, and we will not start until you are comfortable with the set-up.

Do my parents or guardians know about this? (If applicable)

This study information has been given to your parents/guardian and they said that you could take part in the study. You can talk this over with them before you decide**.**

Research Related Injuries

In the event that this research related activity results in an injury, treatment will be made available including first aid, emergency treatment, and follow-up care as needed. Care for such will be billed in the ordinary manner to you or your insurance company/South African public assistance. No reimbursement, compensation, or free medical care is offered by Wayne State University or the University of Cape Town. If you think that you have suffered a research related injury, please contact the Cape Town PI (Dr. Christopher Molteno) right away at 021-406-6291.

What about confidentiality?

Every reasonable effort will be made to keep your records (medical or other) and/or your information confidential, however we do have to let some people look at your study records.

We will keep your records private unless we are required by law to share any information. The law says we have to tell someone if you might hurt yourself or someone else. The study doctor can use the study results as long as you cannot be identified.

The following information must be released/reported to the appropriate authorities if at any time during the study there is concern that:

- child abuse or elder abuse has possibly occurred,
- you disclose illegal criminal activities, illegal substance abuse or violence

What if I have any questions?

For questions about the study please call Dr. Christopher Molteno at 021-406-6291. If you have questions or concerns about your rights as a research participant, the Chair of the Institutional Review Board can be contacted at 001-313-577-1628 or you can contact the Chair of the University of Cape Town Research Ethics Committee at 021-406-6338.

Do I have to be in the study?

You don't have to be in this study if you don't want to or you can stop being in the study at any time. Please discuss your decision with your parents and researcher. No one will be angry if you decide to stop being in the study.

AGREEMENT TO BE IN THE STUDY

Your signature below means that you have read the above information about the study and have had a chance to ask questions to help you understand what you will do in this study. Your signature also means that you have been told that you can change your mind later and withdraw if you want to. By signing this assent form you are not giving up any of your legal rights. You will be given a copy of this form.

_____ _____

Signature of Participant (13 yrs & older) Date

_____

Printed name of Participant (13 yrs & older)

_____ _____

**Signature of Witness (When applicable) Date

_____

Printed Name of Witness

_____ _____

Signature of Person who explained this form Date

_____

Printed Name of Person who explained form

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

Appendix F

Afrikaans Informed Assent Form used for Study III

Dokumentasie van Adolessente Instemming Form
(Ouderdomme 13-17)

Titel: Neurale Basis van Oogknip Kondisionering in FASD

Studie Navorsers: Sandra W. Jacobson, Joseph L. Jacobson,

Christopher D. Molteno, Ernesta M. Meintjes

Hoekom is ek hier?

Hierdie is ‘n navorsingstudie. Slegs mense wat kies om deel te neem word ingesluit by navorsingstudies. Jy word gevra om deel te neem aan hierdie studie omdat jy een van ‘n groot groep kinders is wat al aan hierdie studie deelneem vandat jy gebore is en het deel geneem aan besoeke toe jy ‘n baba was en toe jy 5 jaar oud was. Ons nooi jou uit om deel te neem aan die volgende fase van hierdie studie. Vat asseblief jou tyd om ‘n besluit te neem. Gesels met jou familie daaroor en maak seker om vrae te vra oor enige iets wat jy nie verstaan nie.

Hoekom doen hulle hierdie studie?

Hierdie studie word gedoen om uit te vind hoe kinders dinge leer en onthou en hoe hulle eenvoudige probleme oplos. Ons probeer om te verstaan hoe en of dieet, alkohol, rook, en blootstelling aan dwelms gedurende swangerskap ontwikkeling kan beïnvloed. Ons bestudeer kinders op verskillende ouderdomme met verskillende take om te sien hoe hulle groei en ontwikkel.

Wat sal met my gebeur?

Hier by die Universiteit van Kaapstad, sal ons bestudeer wat gebeur wanneer jy 'n blasie lug in jou oog voel. Jy sal in 'n stoel sit met 'n spesiale helm op jou kop en jy sal 'n video kyk. Elke nou en dan, sal jy 'n lugblasie uit die helm voel kom en soms sal jy 'n geluid hoor. Jy sal ook eenvoudige take doen waartydens jy jou vinger moet tik, prentjies benoem, lyste met woorde leer, lees en somme doen, legkaarte doen, doolhowe doen, geheue en rekenaar take doen en take oor hoe ander mense voel en 'n ander persoon se oogpunt insien. Ons sal jou ook weeg, meet hoe lank jy is, 'n foto neem en kyk hoe goed jy kan sien. Jy sal vanoggend hier spandeer en sal terug kom na die Universiteit van Kaapstad toe op 'n ander dag om die lugblasie taak en die ander take wat ek genoem het te doen.

Die tweede deel van die studie behels neurobeelding, wat ‘n nuwe manier is om van die brein te leer deur prentjies te neem van die brein. Hierdie prentjies kan ons help om beter te verstaan hoe die brein werk. Vir hierdie deel van die studie sal ons jou en jou ma na Tygerberg Hospitaal toe vervoer. Gedurende die neurobeelding, sal jy op ‘n plastiek bed lê wat in ‘n groot masjien inskuif wat ‘n skandeerder genoem word. Ons sal jou vra om so stil as moontlik te lê terwyl die prentjies geneem word. Die afneem van hierdie prentjies (foto’s) van die brein maak nie seer nie en word elke dag deur baie mense in die hospitaal gebruik.
Vir 'n gedeelte van die tyd in die skandeerder sal jy na videos kyk, en vir 'n gedeelte van die tyd sal jy eenvoudige take doen waartydens jy jou vinger moet tik of eenvoudige legkaarte doen, of lees en somme doen, of dinge probeer onthou, of na prentjies kyk en probeer uitwerk of twee mense dieselfde gevoelens voel. Daar sal een sessie in die skandeerder wees.
Ons sal jou ook vra om vir ons ‘n bietjie van jou spoeg (speeksel) te gee en 'n verpleegster sal 'n klein hoeveelheid bloed van jou arm neem om te bestudeer hoe jou gene (familie eienskappe wat jy van jou ouers af kry) beïnvloed hoe jy hierdie take doen en hoe jy optree.

Hoe lank sal ek in die studie wees?

Jy sal twee dae in die studie wees vir hierdie fase, vir ongeveer 3-4 ure by ons laboratorium by die Universiteit van Kaapstad (insluitend ontbyt, 'n peuselhappie, en middagete) en een besoek van sowat 45-50 minute in die skandeerder en 1 uur van opleiding en assessering buite die skandeerder by Tygerberg Hospitaal.

Sal die studie my help?

Jy sal nie daarby baat om in hierdie studie te wees nie, maar inligting uit hierdie studie kan ander mense in die toekoms help om beter te verstaan hoe die brein verskillende take verrig en of dieet, alkohol, rook, of blootstelling aan dwelms gedurende swangerskap beïnvloed hoe die brein werk.
Terwyl jy in hierdie fase van die navorsing deel neem, sal ons vir jou 'n klein geskenkie gee en 'n foto wat van jou brein geneem is aan die einde van die skandering. Ons sal ontbyt, 'n peuselhappie, en middagete voorsien elke keer as jy na ons laboratorium toe kom by die Universiteit van Kaapstad of Tygerberg Hospitaal.

Sal enige iets sleg met my gebeur?

Daar is geen risiko's verbonde aan om in die skandeerder by Tygerberg Hospitaal te wees nie, of enige van die take wat ons met jou doen in ons laboratorium aan die Universiteit van

Kaapstad nie. Die risiko van bloed trek sluit in 'n bietjie tydelike ongemak, swelling en selde infeksie. Hierdie risiko's sal klein wees, want die bloed sal geneem word deur 'n opgeleide persoon (verpleegster/tegnikus).
Sommige mense voel senuweeagtig in ‘n klein beperkte spasie, soos wanneer hulle in die skandeerder is. Jy sal voor die tyd oefen hoe dit gaan voel in 'n oefen skandeerder. Ons sal vir jou oorpluisies of oorfone gee sodat die harde geraas van die skandeerder jou nie pla nie. Daar is 'n knoppie wat jy kan druk om vrae te vra of die skandering te stop op enige tyd. Jy kan te alle tye by die skandeerder uitsien, en ons sal nie begin voordat jy gemaklik is nie.

Weet my ouers of voogde hiervan? (Indien van toepassing)

Hierdie studie inligting is aan jou ouers/voogde gegee en hulle het gesê dat jy kan deel neem aan die studie. Jy kan met hulle hieroor praat voordat jy besluit.

Navorsingsverwante Beserings

Indien hierdie navorsingsverwante aktiwiteite lei tot 'n besering, sal behandeling beskikbaar gemaak word, insluitend eerstehulp, noodbehandeling, en opvolg-sorg soos benodig. Sulke sorg sal betaalbaar wees in die gewone manier deur jou of jou versekerings maatskappy/Suid-Afrikaanse openbare hulp. Geen terugbetaling, vergoeding, of gratis mediese sorg word verskaf deur Wayne State Universiteit of die Universiteit van Kaapstad nie. As jy dink dat jy 'n navorsingsverwante besering opgedoen het, kontak asseblief dadelik die Kaapstad hoofnavorser (Dr Christopher Molteno) by 021-406-6291.

Wat van vertroulikheid?

Elke redelike poging sal aangewend word om jou rekords (mediese of ander) en/of jou inligting konfidensieel te hou, maar ons moet sommige mense na jou studie rekords laat kyk. Ons sal jou rekords geheim hou tensy ons deur die wet vereis word om enige inligting te deel. Die wet sê dat ons iemand moet vertel as jy dalk jouself of iemand anders mag seer maak. Die studie dokter kan die studie resultate gebruik so lank as wat jy nie geïdentifiseer kan word nie.
Die volgende inligting moet vrygelaat word/gerapporteer word aan die toepaslike owerhede indien daar te eniger tyd gedurende die studie kommer is dat:

- kindermisbruik of mishandeling van bejaardes moontlik plaasgevind het,
- jy onwettige kriminele aktiwiteite openbaar, onwettige drank-en dwelmmisbruik, of geweld

Wat as ek enige vrae het?

Vir vrae oor die studie kontak asseblief vir Dr Christopher Molteno by 021-406-6291. Indien jy enige vrae of bekommernisse het oor jou regte as 'n deelnemer aan die navorsing, kan die voorsitter van die Wayne State Universiteit se Menslike Navorsings Komitee gekontak word by 001-313-577-1628 of jy kan die voorsitter van die Universiteit van Kaapstad Navorsings-Etiek Komitee kontak by 021-406-6338.

Moet ek in die studie wees?

Jy hoef nie in hierdie studie te wees as jy nie wil nie of jy kan ophou om in die studie te wees op enige stadium. Bespreek asseblief jou besluit met jou ouers en navorser. Niemand sal kwaad wees as jy besluit om op te hou om in die studie te wees nie.

INSTEMMING OM IN DIE STUDIE TE WEES

Jou handtekening hieronder beteken dat jy die bogenoemde inligting oor die studie gelees het, en dat jy kans gekry het om vrae te vra om jou te help verstaan wat jy in hierdie studie gaan doen. Jou handtekening beteken ook dat daar aan jou verduidelik is dat jy later van besluit mag verander en onttrek as jy wil. Jy gee nie enige van jou regte op deur hierdie vorm te teken nie. Ons sal vir jou 'n kopie van hierdie toestemmingsvorm gee.

_____ _____

Handtekening van Deelnemer (13 j. & ouer) Datum

_____

Naam van Deelnemer in drukskrif (13 j. & ouer)

_____ _____

**Handtekening van Getuie (Wanneer van toepassing) Datum

_____

Naam van Getuie in drukskrif

_____ _____

Handtekening van Persoon wat vorm verduidelik het Datum

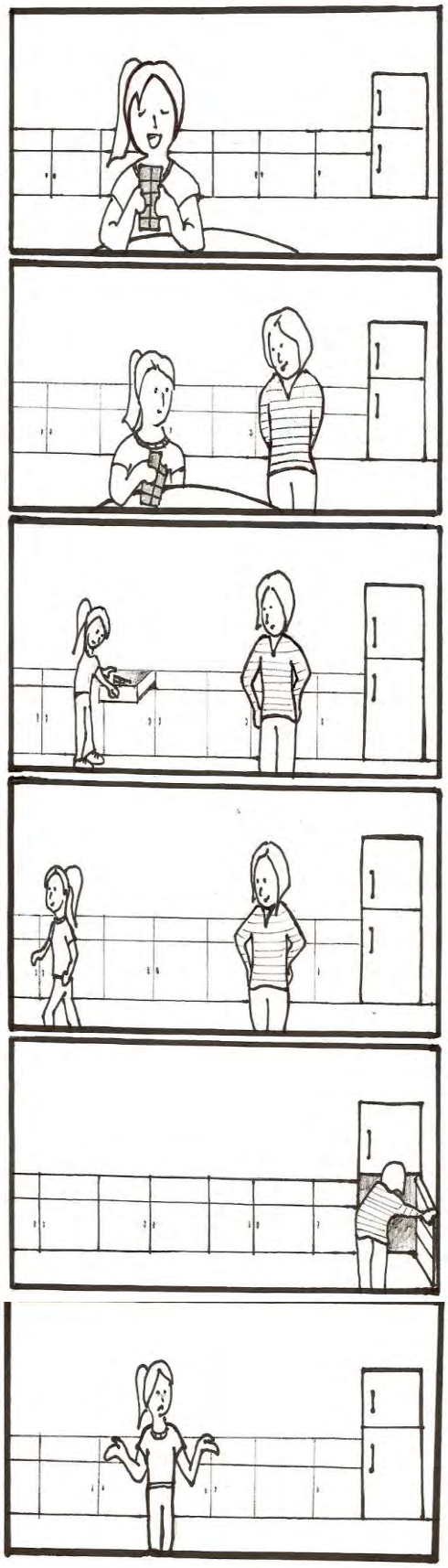
_____

Naam van Persoon wat vorm verduidelik het

**Gebruik wanneer toestemmingsvorm aan deelnemer voorgelees is (bv. wanneer ongeletterd, wetlik blind, vertaal in 'n vreemde taal).

Appendix G

Example of first-order false-belief stimuli



One day, Emma is in the kitchen eating a chocolate bar.

Then, Emma's mom comes in and says, "Emma, put away that chocolate. It is time to do your chores."

"Okay," says Emma, "but when I come back I'm going to finish eating my chocolate."

Emma puts her chocolate in the drawer….

…and leaves to go do her chores.

It is a very hot day. Mom is afraid that the chocolate will melt.

Mom takes the chocolate from the drawer and puts it in the fridge.

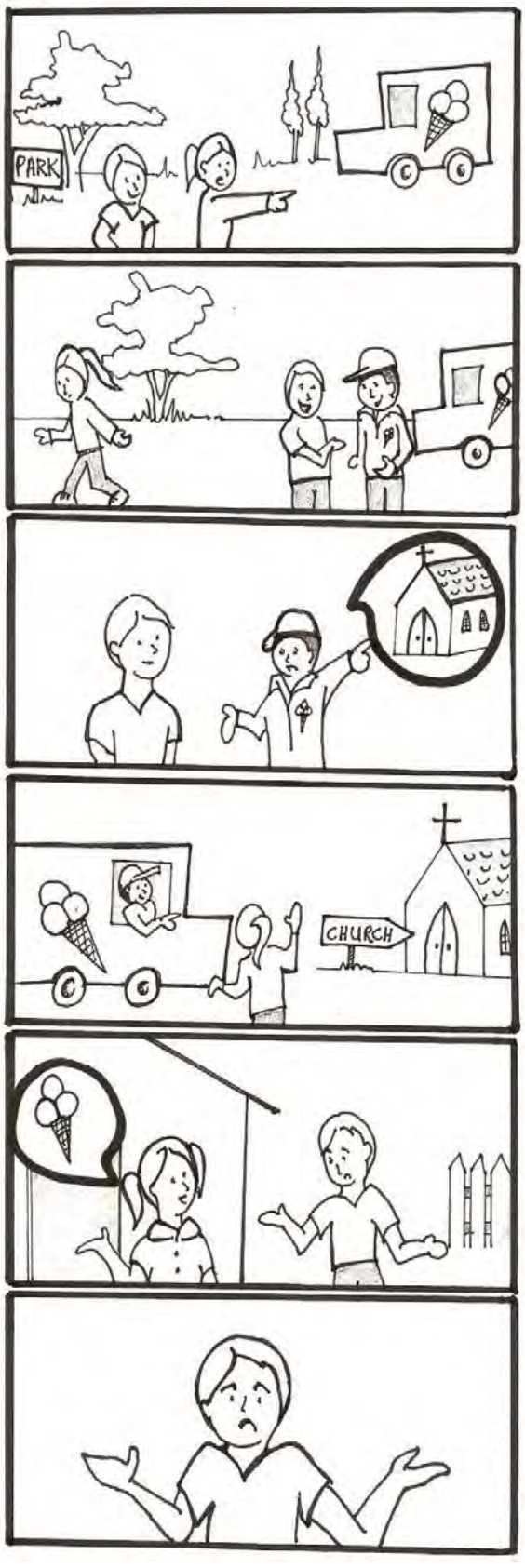
Where did Emma put the chocolate before she went to do her chores? Where is the chocolate now?

Later on, Emma comes back into the kitchen and wants to eat her chocolate.

Appendix H

Example of second-order false-belief stimuli

John and Mary are in the park when they see an ice-cream truck.



Mary would like to buy an ice-cream, but she has no money with her!

The ice-cream man tells Mary to go home and get her money because he will be staying in the park all day.

Mary goes home and John stays in the park.

Then, the ice-cream man tells John that he is moving to the church. He drives off and John goes home.

On his way to the church, the ice-cream man meets Mary. He tells her where he is going, and they arrange to meet at the church so that Mary can get her ice-cream. *Does John know that Mary saw the ice-cream man and arranged to meet him at the Church?*

Later, John goes to Mary's house. Her sister says she has gone to buy ice-cream.

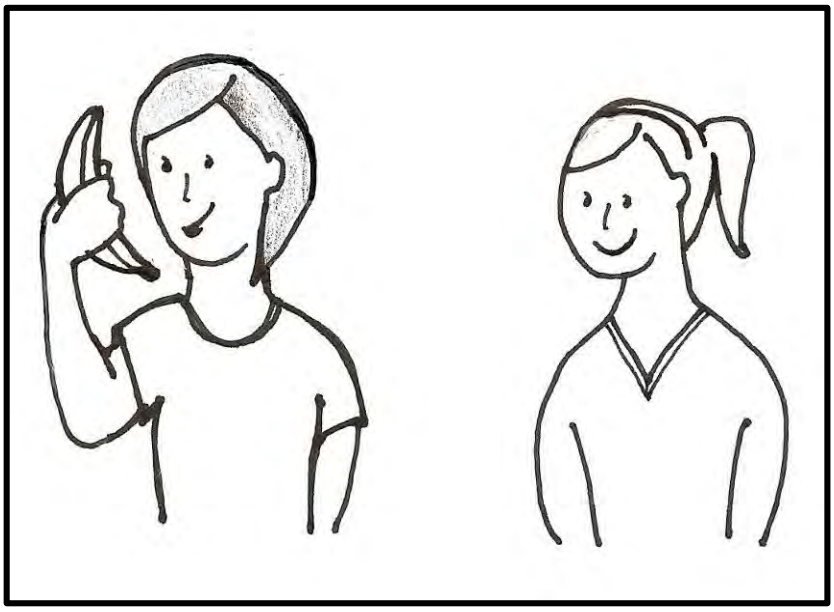
Where will John look for Mary?

Why will he look there?

Appendix I

Example story from the Strange Stories task

Katie and Emma are playing in the house. Emma picks up a banana from the fruit bowl and holds it up to her ear. She says to Katie, “Look! This banana is a telephone!”



Is it true what Emma says?

Why does Emma say this?

Appendix J

Example of a Faux-Pas story

Kim helped her Mum make an apple pie for her uncle when he came to visit. She carried it out of the kitchen. “I made it just for you”, said Kim. “Mmm”, replied Uncle Tom, “That looks lovely. I love pies, except for apple, of course!”

What kind of pie had Kim made?

Did Uncle Tom know that the pie was an apple pie?

Did anyone say something they shouldn’t have said?

Appendix K

Post-hoc analysis of EF tasks

As indicated in the text above, the number of participants completing each EF task varied and details are provided here. All EF tasks administered are listed here with the number of participants from each group included in the analyses presented in parentheses: WISC Digit Span Backwards (FAS: *n* = 12; PFAS: *n* =11; HE: *n* = 27; Controls: *n* = 38); Tower of London (FAS: *n* = 11; PFAS: *n* =11; HE: *n* = 27; Controls: *n* = 35); Rubia Stop (FAS: *n* = 10; PFAS: *n* =9; HE: *n* = 21; Controls: *n* = 30); Stroop Set Shifting/Interference (FAS: *n* = 10; PFAS: *n* =9; HE: *n* = 25; Controls: *n* = 36); Children's Colours Trail Task (FAS: *n* = 11; PFAS: *n* =11; HE: *n* = 27; Controls: *n* = 36); Verbal Fluency Letters (FAS: *n* = 10; PFAS: *n* =9; HE: *n* = 27; Controls: *n* = 36); and Verbal Fluency Categories/Switch (FAS: *n* = 10; PFAS: *n* =10; HE: *n* = 27; Controls: *n* = 36).

One-way analysis of variance showed significant between-group differences on six of the nine EF tasks administered. The three tasks that showed no significant group differences were the Tower of London, the Stroop interference task and the Children's Colour Trails Task. Post-hoc pairwise comparisons using the Fisher LSD analysis was run for all the other EF tasks to determine between which groups the differences exist.

For the Digit span backwards task a significant difference was seen between the FAS and HE (p=.036) and the FAS and control (p=.032) groups, where the FAS group on average had fewer correct trials. Similarly, the performance of the PFAS group was poorer compared to the HE (p=.051) and control (p=.048) group. There were no significant differences in performance between the FAS and PFAS groups and the HE and control group. For the Rubia Stop task there was a significant group differences between the FAS and HE (p=.006) and FAS and control (p=.003) groups indicating that the FAS group takes significantly longer to stop a response. For the Verbal Fluency Letters subtest a significant difference was seen

between the between the FAS and HE (p=.031) group, suggesting that the FAS group gave significantly fewer responses. Similarly, on this task the PFAS group performed significantly worse than both the HE (p=.008) and control (p=.035) group. For the Verbal Fluency Categories task a significant difference was seen between the FAS and HE (p=.028) and the FAS and control (p=.018) group indicating that suggesting that the FAS group gave significantly fewer responses. Similarly, on that same task the PFAS group performed significantly worse compared to both the HE (p=.002) and control (p=.001) group. For the Verbal Fluency Switch task a significant difference was seen between the FAS and HE (p=.001) and the FAS and control (p=.001) group, indicating that suggesting that the FAS group gave significantly fewer responses. Similarly, on that same task the PFAS group performed significantly worse compared to both the HE (p=.005) and control (p=.006) group. Finally, on the Stroop Set Shifting task between a significant difference was shown between the FAS and HE (p=.001) and FAS and control (p=.011) group suggesting that the FAS group takes significantly longer in completing the set shifting task compared to HE and control participants but not compared to the PFAS group.

Appendix L

Correlation matrix for 2-back with potential confounders

Table L1

Correlation matrix for 2-back working memory task and potential confounders, showing all ps > .10

	2-back *d*-prime
Child sex	*r*= .12; *p*=.303
Child age at testing	*r*=-.13; *p*=.256
Maternal smoking	*r*=-.08; *p*=.479
Maternal education	*r*= .11; *p*=.356

Note. r= pearson correlation

Appendix M

Scatterplot showing the relation between the RME and the affective appraisal task

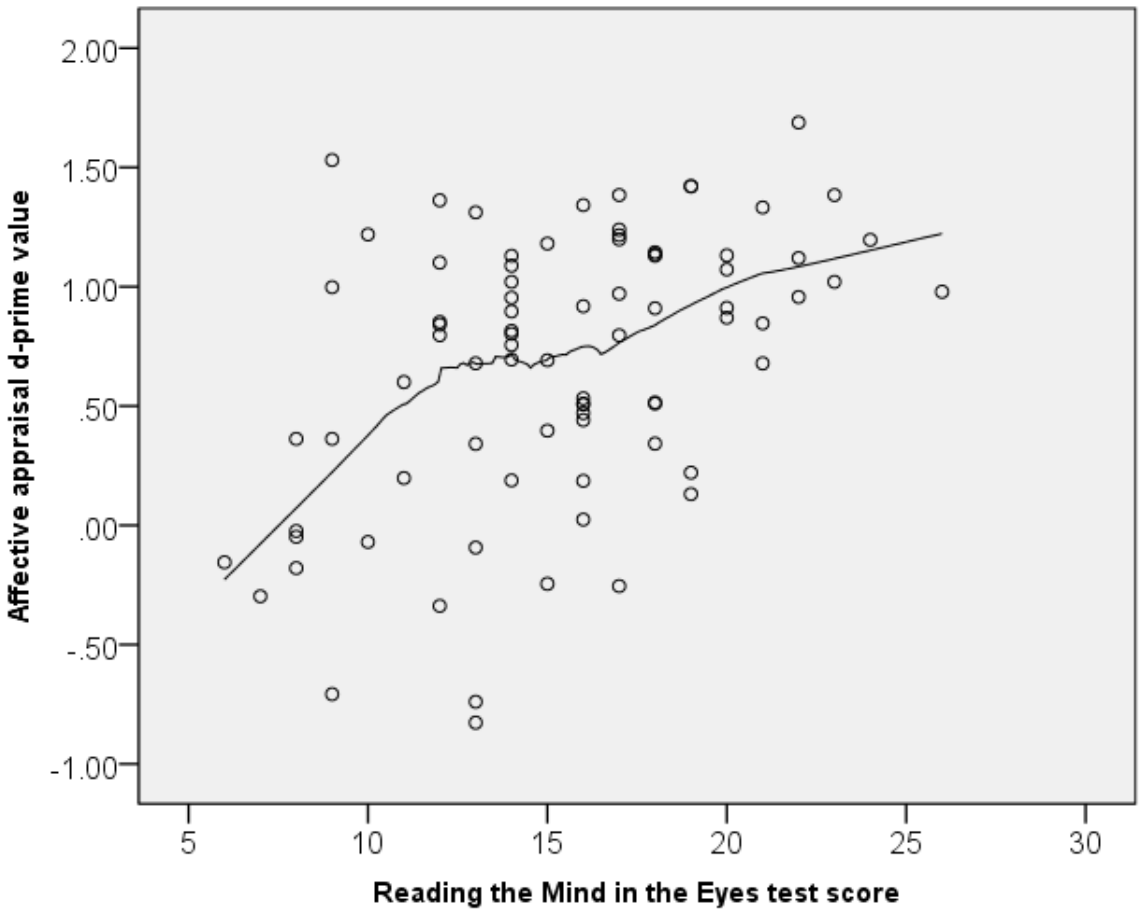


Figure M1. Relation between the RME test and Affective Appraisal

Appendix N

Copy of the CUBIC MRI screening protocol completed prior to each participant's scan

Cape Universities Brain Imaging Centre (CUBIC)

MRI SCREENING FORM

Patient /Participant Information:

Name	Radiographer
Date of Birth	Ward/Clinic (*if applicable*)
Weight	Folder number/Project name

The following information is very important to ensure your safety and to prevent any interference during the MR procedure.

Please answer the following questions (mark with an X):

	Yes	No	Don't know
Pacemaker			
Aneurysm Clip(s)			
Artificial Heart valve			
Vena Cava Filter			
Prosthesis (e.g. eye, breast, hip etc.)			
Shrapnel in eye or body			
Neurostimulator			
Cochlear implant (ear) or Hearing Aid			
? Any other implants (e.g. Screws, plates, joint replacements)			
? Pregnant			
? Previous MRI Investigation with Intravenous Contrast			
Is there any other device implanted or are there any other ailments that you think that we should be aware of?			
In case of Intravenous Injections:			
? Diabetic			
? Renal Impairment			
? Asthma/Respiratory disease			
? Allergies			

I hereby acknowledge that the potential risks of the examination have been explained to me and that during the course of the investigation it may be necessary for the intravenous injection of a contrast agent.

Attention: It is the policy of this institution not to discuss results of the MR investigation with the patients for ethical reasons. All enquiries in this regard should be directed to the referring physician.

Signature: **Date:**

Cape Universities Brain Imaging Centre
Fisan Building, Faculty of Health Sciences, University of Stellenbosch, Tygerberg, 7505
Tel: 27-21-938-9646 Fax: 27-21-938-9728 www.sun.ac.za/cubic

Appendix O

Scanner protocol and fMRI instructions used by examiner

English scanner protocol

MOCK SCANNER

Note: When introducing children to the scanner use book/blurry picture to demonstrate what is said below.

Today you are going to go into a machine that looks like this, it's just a bit bigger. You are going to lie in the tunnel, but only your head is going to go inside. The big machine takes pictures of the inside of your body. If you lie with your stomach in the big machine, it will take a picture of the inside of your stomach (show picture). If you lie with your foot in the big machine, it will take a picture of the inside of your foot (show picture). If you lie with your head in the big machine, it will take a picture of your brain (show picture). Because you are going to be lying with your head inside the big machine, we are going to take pictures of your brain today. It doesn't hurt you at all, it is just like a camera that takes photos. Have you ever had a photo taken before? It did not hurt you hey? Just like a camera, if you move during a picture it is going to be blurry. So you need to lie very still when the machine is busy taking pictures. You will know that the machine is busy taking pictures when it is very noisy. Just now we will give you a chance to lie in a practice machine and listen to what the sounds are like. When you are in the big machine and you hear the noises you will need to lie very still, because it is taking pictures. When we are all finished today, you will get a picture of your brain!

When you are in the big machine you will get to wear earphones so that you can hear the movie. We will also be talking to you the whole time--We will remind you to lie still. You will lie on your back and watch the movie and then you will play some games. You will have something like this (show computer mouse) on your stomach to help you play the games. We will practice the games here before you go into the big machine. The games that we practice here will be shorter than the games you play when you are in the big machine. After we practice the games, we will practice staying still in a pretend machine.

Note: Before running practice tasks, make sure that the NumLock key is on. You will have to press the NumLock key once the task has been opened.

PRACTICE AFFECTIVE APPRAISAL

'Faces Game'

This next game will be the second game that you play in the big machine. You are going to see faces of different people coming up on the screen one at a time and you have to figure out how each one feels. Each time you see a face, I want you to decide if the person on the screen feels the same as the person you saw just now or different than the person you saw just now. It is not about whether it is the same person or if they look alike, but it is about whether he or she feels the same as the one before or not. It does not have to be the same person. You just need to figure out if he or she feels the same as the person before or not. Sometimes you might see the same person again, and then you also just need to decide if he or she is feeling different than before or not. Sometimes you will see a picture of a face that has been scrambled. I want you to treat these faces in the same way, are they the same or different to the face just before?

Administer paper practice trial.

Now, when we play this game on the computer you need to press this button with your pointer finger when you think the person is feeling the same (5); and this button with your middle finger (6) when you think the person is feeling different. Between each picture there will be crosses like this one. This is where you should be looking on the screen. When you see the cross, just wait for the next picture to show.

Administer paper practice trial with button presses.

Administer computer practice trial.

- **Double click on Affective Appraisal shortcut on the desktop**
- **Check that NumLock key is on**
- **Enter Subject ID and Session Number (1)**

Note: It is very important that the child keep their hand on the keys when they practice the task. Their hand will be on the response box throughout the scan and they will not be able to see the buttons. Also, make sure that the child is not relying on saying 'same' or 'different' out loud—they *cannot* speak during the task in the scanner.

When you play this game in the big magnet, you are going to have to press the buttons with your right pointer finger when you think the person is feeling the same and your right middle finger when you think the person is feeling different.

ONCE PRACTICE IS COMPLETED:

Good job! When you are in the big machine you will see three sets of different pictures and we want you to try your best to remember all of them. Remember that you have to press the buttons with your pointer finger if it is an inside picture and your middle finger if it is an outside picture. We want you to know this is going to be tricky, but try your best to remember all of the pictures you see. But remember,
you will play the memory game in here after you are all done in the big machine.

We will go over the directions one more time when you are in the magnet, but it might be hard to hear so it's important to ask any questions right now if you aren't sure how to play one of the games. Do you have any questions?

Ok, let's go over the order of the games once more! During the first 15 minutes that you are in the big machine you just need to lie very still. After that, your first game in the big machine will be the picture game, then you will play the faces game, next you will see three sets of inside and outside pictures and it's important to try your best to remember all of them. When you are all done in the big machine you are going to be tested on your memory of the inside and outside pictures.

Do you have any questions?

PRACTICE MOCK SCAN

Would you like to feel what it is like to lie inside the machine? First they will give you ear plugs and then you will put some earphones on. You are going to lie on your back and have a thing like this (use computer mouse) that will be on your tummy and you are going to have to press the buttons like we practiced just now. You will also have a thing like this above your head. We put this thing on because it has a small mirror inside it. The mirror will show you what is behind you and will let you see the movie. Can you see the picture on the wall now?

While you are in the big machine we will talk to you. We will ask you questions and then you must answer 'yes' or 'no' but without moving your head. Let's practice. Is your name___? Are you ____ years old? Are you lying still? Remember, you must try not to talk in the big machine. If you talk your head moves and then the picture is blurry.

Encourage the child to respond without moving their head

You are going to lie in the big machine and you will hear different noises. Remember, when you hear the loud noises it means that the machine is taking pictures and that you will need to lie very still. Let's listen to some of the noises.

Play noises from Laptop. Make sure that speakers are turned on.
While the noises are playing reassure the child that the noises don't hurt them and remind them to lie still in the big machine.

Note: It is very important to provide affirmation rather than asking the child if they are ok/nervous? Rather say something like "you are doing great!" Also, make sure that the child is as relaxed as possible. They will feel m much calmer if their rapport with us is good!

Allow the child to choose a movie.

fMRI SCAN

AFFECTIVE APPRAISAL

'Faces Game'

Hi (name). Great Job! Now we are going to play the faces game. I want you to look carefully at each face and decide whether the person is feeling the same as the one before or different than the one before. Remember, it is not about whether it is the same person or if they look alike, but it is about whether he or she feels the same as the one before or not. If you think that they are feeling the same, I want you to press the button with your pointer finger; if you think that they are feeling different, I want you to press the button with your middle finger. Press the button that you must press if they feel the same. Good! Now press the button you must press if they feel different. Good! Remember, you must press the button for same or different every time you see a face. Are you comfortable and ready to start?

Administer task:

- Press 'run' button

- Enter Subject ID and Session Number (1)

Afrikaans scanner protocol

MOCK SCANNER

Note: When introducing children to the scanner use book/blurry picture to demonstrate what is said below.

Vandag gaan jy in ‘n groot masjien in wat lyk soos hierdie, maar wat net ‘n bietjie groter is. Jy gaan in ‘n tonnel lê, maar net jou kop gaan binne in. Die groot masjien neem foto’s van die binnekant van jou lyf. As jy met jou maag binne die groot masjien lê, sal dit ‘n foto van die binnekant van jou maag neem (show picture). As jy met jou voet in die groot masjien lê, sal dit ‘n foto van die binnekant van jou voet neem (show picture). As jy met jou kop in die groot masjien lê, sal dit ‘n foto van jou brein neem (show picture). Omdat jy vandag met jou kop in die groot masjien gaan lê, gaan ons foto’s van jou brein neem. Dit maak glad nie seer nie, dis net soos ‘n kamera wat foto’s neem. Het iemand al ooit ‘n foto van jou geneem? Dit was nie seer nie, nê? Net soos ‘n kamera, as jy beweeg trewyl die foto geneem word, gaan dit onduidelik uitkom. So jy moet baie stil lê terwyl die masjien foto’s neem. Jy sal weet dat die masjien foto’s neem wanneer dit ‘n groot geraas maak. Ons sal jou nou nou kans gee om in die oefen masjien te lê en te hoor hoe die geluide klink. Wanneer jy in die groot masjien is, en jy hoor die geluide, gaan jy baie stil moet lê want dan is dit besig om foto’s te neem.

Wanneer ons heeltemal klaar maak vandag, sal jy ‘n foto van jou brein kry!
Wanneer jy in die groot masjien is, sal jy oorfone dra sodat jy die fliek kan hoor. Ons sal ook die heeltyd met jou gesels – Ons sal jou herinner om stil te lê. Jy gaan op jou rug lê en fliek kyk en dan gaan jy ‘n paar speletjies speel. Jy sal iets soos hierdie hê (show computer mouse) wat op jou maag gaan lê om jou te help om die speletjies te speel. Ons sal die speletjies hier oefen voordat jy in die groot masjien ingaan. Die speletjies wat ons hier gaan oefen sal korter wees as die speletjies wat jy gaan speel as jy in die groot masjien is. Nadat ons die speletjies geoefen het, sal ons oefen hoe om stil te lê in die groot masjien.

Note: Before running practice tasks, make sure that the NumLock key is on. You will have to press the NumLock key once the task has been opened.

PRACTICE AFFECTIVE APPRAISAL

'Gesigte Speletjie'

Die volgende speletjie sal die tweede speletjie wees wat jy in die groot masjien gaan speel. Jy gaan gesigte van verskillende mense op die skerm sien opkom, een op 'n slag, en jy gaan moet besluit hoe elkeen van hulle voel. Elke keer as jy 'n gesig sien, wil ek hê jy moet besluit of die mens op die skerm dieselfde voel as die mens wat jy net voor dit gesien het, of anders voel as die mens wat jy net voor dit gesien het. Dit gaan nie daaroor of dit dieselfe mens is nie, en ook nie of hulle dieselfde lyk nie. Dit gaan daaroor of die mens dieselfde voel as die een voor hom of haar. Dit hoef nie dieselfde mens te wees nie. Jy moet net uitwerk of hy of sy dieselfde voel, of anders voel, as die vorige mens. Soms mag jy dalk dieselfde mens weer sien, en dan moet jy ook net besluit of hy of sy dieselfde of anders voel as van tevore. Soms sal jy 'n prentjie van 'n gesig sien wat op geskommel is. Ek wil hê jy moet hierdie gesigte dieselfde hanteer as die ander, is hulle dieselfde of anders as die gesig net voor dit?

Administer paper practice trial.

Nou, wanneer ons die speletjie op die rekenaar speel, moet jy hierdie knoppie met jou wysvinger druk as jy dink die mens voel dieselfde (5); en hierdie knoppie met jou middelvinger (6) as jy dink die mens voel anders. Tussen elke prentjie sal daar 'n kruisie wees, soos die een. Dit wys waar jy moet kyk op die skerm. As jy die kruisie sien, wag maar net vir die volgende prentjie om te wys.

Administer paper practice trial with button presses.

Administer computer practice trial.

- **Double click on Affective Appraisal shortcut on the desktop**
- **Check that NumLock key is on**
- **Enter Subject ID and Session Number (1)**

Note: It is very important that the child keep their hand on the keys when they practice the task. Their hand will be on the response box throughout the scan and they will not be able to see the buttons. Also, make sure that the child is not relying on saying 'dieselfde' or 'anders' out loud—they *cannot* speak during the task in the scanner.

Wanneer jy hierdie speletjie in die groot magneet speel, gaan jy die knoppies met jou regter wysvinger moet druk as jy dink die mens voel dieselfde, en met jou regter middelvinger as jy dink die mens voel anders.

PRACTICE MOCK SCAN

Wil jy voel hoe dit voel om in die masjien te lê? Eerste sal hulle vir jou oorpluisies gee en dan sal jy oorfone op jou ore sit. Jy gaan op jou rug lê en iets soos hierdie hê (use computer mouse) wat op jou maag lê en dan gaan jy die knoppies moet druk net soos wat ons nou geoefen het. Jy sal ook iets soos hierdie bo jou kop hê. Ons sit hierdie ding op, want hy het 'n klein spïeeltjie aan die binnekant. Die spïeeltjie sal jou help om te sien wat agter jou is en sal jou ook die fliek kan laat sien. Kan jy nou die prentjie op die muur sien?
Terwyl jy in die groot masjien is sal ons met jou gesels. Ons sal vir jou vrae vra en dan sal jy 'ja' of 'nee' moet antwoord, maar sonder om jou kop te beweeg. Kom ons oefen. Is jou naam___? Is jy ____ jaar oud? Lê jy stil? Onthou, jy moet probeer om andersins nie te praat in die groot masjien nie. As jy praat, dan beweeg jou kop en die foto kom onduidelik uit.

Encourage the child to respond without moving their head

Jy gaan in die groot masjien lê en dan sal jy verskillende geluide hoor. Onthou, wanneer jy die harde geluide hoor beteken dit dat die masjien besig is om foto's te neem en dat jy baie stil moet lê. Luister na van die geluide wat jy gaan hoor.

Play noises from Laptop. Make sure that speakers are turned on.
While the noises are playing reassure the child that the noises don't hurt them and remind them to lie still in the big machine.

Note: It is very important to provide affirmation rather than asking the child if they are ok/nervous? Rather say something like "you are doing great!" Also, make sure that the child is as relaxed as possible. They will feel much calmer if their rapport with us is good!

Allow the child to choose a movie.

fMRI SCAN
AFFECTIVE APPRAISAL

'Faces Game'

Haai (naam). Goeie werk! Nou gaan ons die gesigte speletjie speel. Ek wil he jy moet versigtig na elke gesig kyk en besluit of die mens dieslefde voel as die vorige een of anders as die vorige een. Onthou, dit gaan nie daaroor of dit dieslefde mens is, en of hulle dieselfde lyk nie, maar dit gaan daaroor of hy of sy dieslfde voel as die vorige mens. As jy dink dat hulle dieselfde voel, wil ek hê jy moet die knoppie met jou wysvinger druk; as jy dink dat hulle anders voel, wil ek hê jy moet die knoppie met jou middelvinger druk. Druk nou die knoppie wat jy sal druk as hulle dieselfde voel. Mooi! Druk nou die knoppie wat jy sal druk as hulle anders voel. Baie mooi! Onthou, jy moet elke keer wanneer jy 'n gesig sien die knoppie druk vir dieselfde of anders. Is jy gemaklik en reg om te begin?

Administer task:

- **Press 'run' button**
- **Enter Subject ID and Session Number (1)**

Appendix P

Table showing between group comparison of regions of interest network showing greater activation for happy faces compared to angry faces after

Table P1.

Between group comparison of regions of interest network showing greater activation for happy faces compared to angry faces after cluster size correction (threshold p<.05; cluster sizes of 7.8 – 156.2 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group	No significant differences		
Control group > HE group	No significant differences		
FAS/PFAS group > Control group			
Frontal			
R frontal gyrus, middle	34,20,36	4397	4.30
L frontal gyrus, middle	-40,14,40		3.83
R frontal gyrus, medial	16,40,18	42	2.35
L frontal gyrus, inferior	-52,30,18	337	3.69
L frontal gyrus, medial	-44,54,-8		2.82
Occipital			
R fusiform gyrus	28,-56,-14	455	3.51
R cuneus	16,-86,10	150	2.63
R lingual gyrus	16,-88,-2		2.61
L lingual gyrus	-16,-92,-6	23	3.39
L fusiform gyrus	-24,-66,-16	439	2.96
Temporal			
R fusiform gyrus	50,-46,-18	455	3.06
R temporal gyrus, inferior	40,-6,-32		2.67
L fusiform gyrus	-58,-14,-28	131	3.02
L temporal gyrus, middle	-44,0,-30		2.92
Limbic			
R parahippocampal gyrus	26,-32,-22	27	2.51
L parahippocampal gyrus	-30,2,-20	67	3.36
L uncus	-18,-4,-24		2.28
Sub-lobar			
L extra-nuclear	-12,-2,8	2956	3.94
R extra-nuclear	26,18,2		3.85
FAS/PFAS group > HE group			
Frontal			
R frontal gyrus, middle	34,20,38	148	3.11
R frontal gyrus, inferior	48,10,38		2.11
L frontal gyrus, middle	-32,46,28	91	2.59

L frontal gyrus, medial	-8,50,12		96	2.49
Occipital				
R lingual gyrus	16,-92,-6		40	2.74
R cuneus	6,-86,4		49	2.33
L cuneus	-2,-78,8			2.11
Sub-lobar				
R extra-nuclear	20,10,14		69	3.32
L extra-nuclear	-16,0,16		68	2.22
HE group > Control group				
Frontal				
R frontal gyrus, inferior	54,14,28		370	3.64
R frontal gyrus, precentral	60,0,26			3.18
R frontal gyrus, middle	32,58,4		272	2.76
R frontal gyrus, medial	10,26,-14			2.13
		18		
L frontal gyrus, precentral	-50,2,40		701	4.10
L frontal gyrus, inferior	-46,2,24			3.77
L frontal gyrus, medial	-6,52,42		837	3.54
L frontal gyrus, superior	-16,54,28			3.46
R frontal gyrus, superior	12,52,28			2.90
L frontal gyrus, middle	-42,52,6			3.41
		430		
Occipital				
R cuneus	20,-78,10		35	3.15
Temporal				
L sub-gyral	-36,-30,-8		25	4.10
Limbic				
R uncus	28,0,-26		1028	3.33
Sub-lobar				
R extra-nuclear	12,-6,16		102	2.13
L caudate-head	-14,22,0		373	3.18
HE group > FAS/PFAS group				
Frontal				
L frontal gyrus, medial	-6,52,42		29	2.40
Sub-lobar				
L extra-nuclear	-26,-36,10		12	2.71

Note. Submaxima within clusters are shown under maxima in italics
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

Appendix Q

Table showing between group comparison of regions of interest network showing greater activation for happy faces compared to sad faces

Table Q1.

Between group comparison of regions of interest network showing greater activation for happy faces compared to sad faces after cluster size correction (threshold p<.05; cluster sizes of 5.9 – 168.2 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group	No significant differences		
Control group > HE group	No significant differences		
FAS/PFAS group > Control group			
Frontal			
R frontal gyrus, middle	34,22,34	430	3.86
R frontal gyrus, superior	34,64,-2	79	2.05
R frontal gyrus, inferior	52,44,0	62	2.57
L frontal gyrus, middle	-44,50,6	114	2.87
L frontal gyrus, inferior	-44,42,0		2.80
Occipital			
L lingual gyrus	-24,-72,-16	168	2.89
R fusiform gyrus	24,-80,-18	54	1.90
Temporal			
R sub-gyral	38,-30,-8	36	2.66
L sub-gyral	-36,-34,-2	39	2.40
Limbic			
R parahippocampal gyrus	20,-4,-22	50	2.38
Sub-lobar			
L caudate body	-14,6,20	264	2.14
L caudate tail	-34,-28,-8	39	2.49
R caudate body	14,10,12	388	2.68
R caudate head	12,20,0		2.54
FAS/PFAS group > HE group			
Frontal			
R frontal gyrus, middle	48,12,34	177	2.90
Occipital			
R fusiform gyrus	26,-78,-18	45	3.28
Limbic			
R anterior cingulate	10,40,2	212	2.26
L anterior cingulate	-6,46,12		2.19

HE group > Control group			
Frontal			
R frontal gyrus, inferior	62,12,26	143	3.43
R precentral gyrus	56,2,40		3.14
R frontal gyrus, superior	34,34,38	183	2.78
R frontal gyrus, middle	32,24,34		2.29
L frontal gyrus, middle	-46,46,16	478	4.83
L frontal gyrus, inferior	-44,42,0		3.17
Occipital			
R lingual gyrus	10,-88,-2	106	2.73
L cuneus	-10,-88,6		2.41
Temporal			
L fusiform gyrus	-44,-52,-24	60	2.56
HE group > FAS/PFAS group			
Frontal			
L frontal gyrus, inferior	-42,44,12	69	2.86
Limbic			
L anterior cingulate	-8,20,26	59	2.54

Note.

Submaxima within clusters are shown under maxima in italics

The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.

Appendix R

Table showing between group comparison of regions of interest network showing greater activation happy faces compared to fearful faces

Table R1.

Between group comparison of regions of interest network showing greater activation happy faces compared to fearful faces after cluster size correction (threshold p<.05; cluster sizes of 8.2-139.5 voxels, voxel size = 2x2x2)

Region	MNI coordinates (x,y,z)	Number of voxels	Peak *t*
Control group > FAS/PFAS group	No significant differences		
Control group > HE group	No significant differences		
FAS/PFAS group > Control group			
Frontal			
R frontal gyrus, middle	34,22,34	170	3.27
L frontal gyrus, superior	-24,62,0	226	3.00
L frontal gyrus, middle	-26,64,12		2.45
L frontal gyrus, medial	-4,48,32	259	2.97
L frontal gyrus, precentral	-46,-6,24	89	2.81
L frontal gyrus, inferior	-52,4,32		2.13
Temporal			
L fusiform gyrus	-60,-12,-28	72	3.16
Occipital			
L fusiform gyrus	-32,-50,-14	79	2.88
R fusiform gyrus	26,-56,-14	58	2.86
R cuneus	16,-86,8	68	2.53
Sub-lobar			
R caudate-tail	36,-26,-8	33	2.60
R putamen	16,12,-10	222	2.55
FAS/PFAS group > HE group	No significant differences		
HE group > Control group			
Frontal			
R frontal gyrus, inferior	60,10,28	908	3.35
R frontal gyrus, superior	26,32,38		3.33
R frontal gyrus, middle	-30,34,42	200	3.29
L frontal gyrus, inferior	-46,0,24	515	4.84
L frontal gyrus, precentral	-34,2,30		2.89
L frontal gyrus, middle	-46,46,16	459	3.50
L frontal gyrus, superior	-8,52,38	286	2.91

Temporal			
R fusiform gyrus	36,-56-18	260	2.72
L fusiform gyrus	-46,-50,-22	310	2.84
L caudate-tail	-36,-28,-8	27	3.02
Occipital			
R fusiform gyrus	34,-66,-14	260	2.91
Sub-lobar			
R putamen	20,0,4	908	3.63
L putamen	-24,10,-4	565	2.67
HE group > FAS/PFAS group			
Frontal			
R frontal gyrus, middle	40,12,34	85	2.66
R frontal gyrus, precentral	40,12,34		2.64
R frontal gyrus, superior	22,58,2	42	1.76
L frontal gyrus, middle	-54,18,32	194	3.56
L frontal gyrus, precentral	-48,2,28		2.97
L frontal gyrus, inferior	-48,6,40		2.35
Temporal			
R fusiform gyrus	38,-58,-18	66	2.15
L fusiform gyrus	-44,-62,-20	141	2.55
L temporal gyrus, inferior	-52,-62,-16		2.51
Sub-lobar			
R putamen	30,-22,4	118	2.51
R lateral globus pallidus	26,-12,-2		2.29

Note. Submaxima within clusters are shown under maxima in italics
The '>' sign indicates that activation of a specific region was greater for the group on the left when contrasted with the group on the right.