FOETAL TESTOSTERONE, COGNITIVE SEX DIFFERENCES AND AUTISTIC TRAITS

BY

BONNIE AUYEUNG

TRINITY COLLEGE

DISSERTATION SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

SCHOOL OF CLINICAL MEDICINE

IN THE DEPARTMENT OF PSYCHIATRY

AT THE UNIVERSITY OF CAMBRIDGE

UNITED KINGDOM

PROFESSOR SIMON BARON-COHEN, SUPERVISOR

1 MAY 2008

Brief Table of Contents

Table of Contents

Preface

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text.

No part of this dissertation has been submitted for any other degree, diploma or other qualification at any other university or institution.

This manuscript does not exceed the 60,000 maximum word limit set by the Degree Committee of Clinical Medicine and Clinical Veterinary Medicine, University of Cambridge.

Acknowledgements

Firstly, I would like to thank my supervisor, Professor Simon Baron-Cohen for his unwavering support and the creative freedom that he has always provided. It has been a great pleasure to work with Simon who has always helped to push me to achieve my potential and has been so generous with his time and assistance.

I would like to thank my colleagues at the Autism Research Centre for all the professional support and friendship that I have received over the years. Thanks must also be given to Jenny Hannah and Paula Naimi who have both provided superb administrative and personal support throughout my stay at the Autism Research Centre. I would also like to thank Emma Ashwin and Rebecca Knickmeyer for the collaborative assistance that they have provided. Emma and Rebecca have also been examining the effects of FT on later child development, and together we have been able to collect far greater amounts of data than it would have been possible alone. A special word of thanks goes to Carrie Allison who has always made herself available to answer the smallest of questions, and her unwavering and constant assistance with life events (not to mention data collection and analysis) have been invaluable.

Many thanks go to Professor Melissa Hines, Dr Rosa Hoekstra, Dr Bhismadev Chakrabarti, Malcolm Bang, Dr Greg Davis, Dr Jag Ahluwalia, Lynn Thomas, Dr Gerald Hackett, Kevin Taylor and Professor Ieuan Hughes for valuable discussions.

I am very grateful to the families who have taken part in this longitudinal study over many years. Without these willing families, the research would not be possible. I am also grateful to Trinity College, Cambridge for supporting me throughout my PhD. Equal thanks go to the Medical Research Council and the Nancy Lurie-Marks Family Foundation who supported the research projects.

Last, but definitely not least, I am grateful to Alex Quayle for his unwavering support, exceptional skills and unlimited enthusiasm for learning and teaching. I am also thankful to my parents Ken and Marian Auyeung, my siblings Azshwee Willden and John Auyeung and my best friend Heidi Kreis for being my biggest supporters.

It is because of these people that I am here, whole and just as excited to continue to navigate through life as I ever have been.

Abbreviations

2D:4D: Second to fourth digit ratio

AQ: Autism Spectrum Quotient

AQ-Child: Autism Spectrum Quotient-Children's Version

AS/HFA: Asperger syndrome/High-Functioning autism

ASC: Autism Spectrum Conditions

BSRI: Bem Sex Role Inventory

CAS-P: Children's Aggression Scale-Parent version

CAST: Childhood Autism Spectrum Test

CBC-A: Child Behavior Checklist-Aggression Subscale

E: Empathising

EFT: Children's Embedded Figures Test

EMB: Extreme Male Brain

EQ: Empathising Quotient

EQ-C: Children's Empathising Quotient

FT: Foetal Testosterone

FO: Foetal Oestradiol

F: Bem Sex Role Inventory Femininity Score

M: Bem Sex Role Inventory Masculinity Score

MR: Mental rotation

PSAI: Pre-School Activities Inventory

Q-CHAT: Quantitative Checklist for Autism in Toddlers

NT: Neonatal Testosterone

S: Systemising

SQ: Systemising Quotient

SQ-C: Children's Systemising Quotient

Abstract

Autism Spectrum Conditions (ASC) are characterised by social and communicative impairment, alongside narrow interests and repetitive behaviour. The incidence of ASC is strongly biased towards males, though the cause of this sex difference remains a topic of debate. Sex differences are found in human social behaviour, and it has been suggested that ASC may be an extreme of the male-typical brain. Prenatal hormones such as foetal testosterone (FT) are known to influence sex-typical behaviour. This thesis reports studies testing which behaviours are associated with FT levels in typically developing children and if there is any correlation between FT and autistic traits.

Chapter 1 first reviews the extreme male brain hypothesis of ASC. It then reviews the role of hormones in sexual differentiation, and methodologies for the investigation of FT effects on postnatal behaviour. Chapter 2 examines whether amniotic FT levels are related to Mental Rotation, Embedded Figures and Targeting, all chosen because these show sex differences (male advantage). FT levels were found to be *positively* related to Embedded Figures score in both boys and girls, but not Mental Rotation or Targeting scores. Chapter 3 investigates if FT is related to childhood gender-related behaviour, finding a *positive* association between FT and male-typical scores on a questionnaire measuring sex-typical play. In a further measure of other sex-typical traits, a positive association was further observed between FT and masculinity scores. Chapter 4 examines if FT levels influence scores on two measures of aggression. Results showed no sex differences or associations for either of these measures.

In Chapter 5, the development of children's versions of the Empathising (EQ) and Systemising Quotients (SQ) are reported and typical sex differences are investigated. Results confirm patterns found in adults (higher EQ in females, higher SQ in males). Children with ASC fitted a 'hyper-masculinised' profile, irrespective of sex. A further investigation showed FT was *positively* correlated with SQ and *negatively* correlated with EQ. Chapter 6 directly investigates links between FT levels and autistic traits. FT levels were *positively* associated with scores on the Childhood Autism Spectrum Test (CAST)

and an adapted version of the Autism Spectrum Quotient for children (AQ-Child). Chapter 7 revealed a similar *positive* correlation between FT and the Quantitative Checklist for Autism in Toddlers (the Q-CHAT). No correlation was found between Q-CHAT score and neonatal testosterone or prenatal oestradiol.

Overall, these studies support a role for FT in the development of some but not all sexually dimorphic behaviours, and in the number of autistic traits a child has. These studies may help further our understanding of the function of FT and point to the need for direct testing of FT in children who later develop ASC.

List of Figures

Chapter 6: Foetal testosterone and autistic traits

Chapter 7: Autistic traits in toddlers

Chapter 8: General Discussion

List of Tables

Chapter 6: Foetal testosterone and autistic traits

Chapter 7: Autistic traits in toddlers

Chapter 8: General Discussion

Chapter 1: Introduction

Autism Spectrum Conditions may be an extreme of the male brain. This chapter reviews the background of the extreme male brain hypothesis, the role of foetal testosterone in sexual differentiation, and the methodologies available for the investigation of the relation between prenatal testosterone and postnatal behaviour in human populations.

1.1. Autism Spectrum Conditions

Many neurodevelopmental conditions occur in males more often than females. These include autism, dyslexia, attention-deficit hyperactivity disorder (ADHD) and early-onset persistent antisocial behaviour (Rutter, Caspi & Moffitt, 2003). Autism in particular has been described as an extreme manifestation of some sexually dimorphic traits or an "extreme male brain" (Baron-Cohen, 2002). In this chapter, the reasons why this condition has been viewed in this light, and the evidence related to it will be reviewed.

Autism, High-Functioning autism, Asperger syndrome and Pervasive Developmental Disorder (not otherwise specified; PDD/NOS) are thought to lie on the same continuum, and can be referred to as Autism Spectrum Conditions (ASC). The American Psychiatric Association uses the term Autism Spectrum Disorders. The use of the term ASC is preferred since those at the higher-functioning end of the autistic spectrum do not necessarily see themselves as having a 'disorder', and the profile of strengths and difficulties in ASC can be conceptualised as atypical but not necessarily disordered. ASC remains a medical diagnosis, hence the use of the term 'condition', which signals that such individuals need support. Use of the term ASC is more respectful to differences, recognises that the profile in question does not fit a simple 'disease' model but includes areas of strength (e.g. in attention to detail) as well as areas of difficulty and does not identify the individual purely in terms of the latter.

ASC are characterised by impairments in reciprocal social interaction and in verbal and nonverbal communication, alongside strongly repetitive behaviours and unusually narrow interests (APA, 1994). Recent epidemiological studies have shown that as many as 1% of people could have an ASC (Baird et al., 2006). The incidence of ASC is strongly biased towards males (Bryson & Smith, 1998; Fombonne, 2005; Tidmarsh & Volkmar, 2003) with a male:female ratio of 4:1 for classic autism (Chakrabarti & Fombonne, 2005) and as high as 8:1 for Asperger Syndrome (Scott et al., 2002a). The

cause of the observed sex difference in ASC also remains a topic of debate. It is possible that males have a lower threshold for expressing the condition (Kraemer, 2000). ASC have a strong neurobiological and genetic component (Stodgell, Ingram & Hyman, 2001), however the specific factors that are responsible for the higher male incidence in ASC are still unclear.

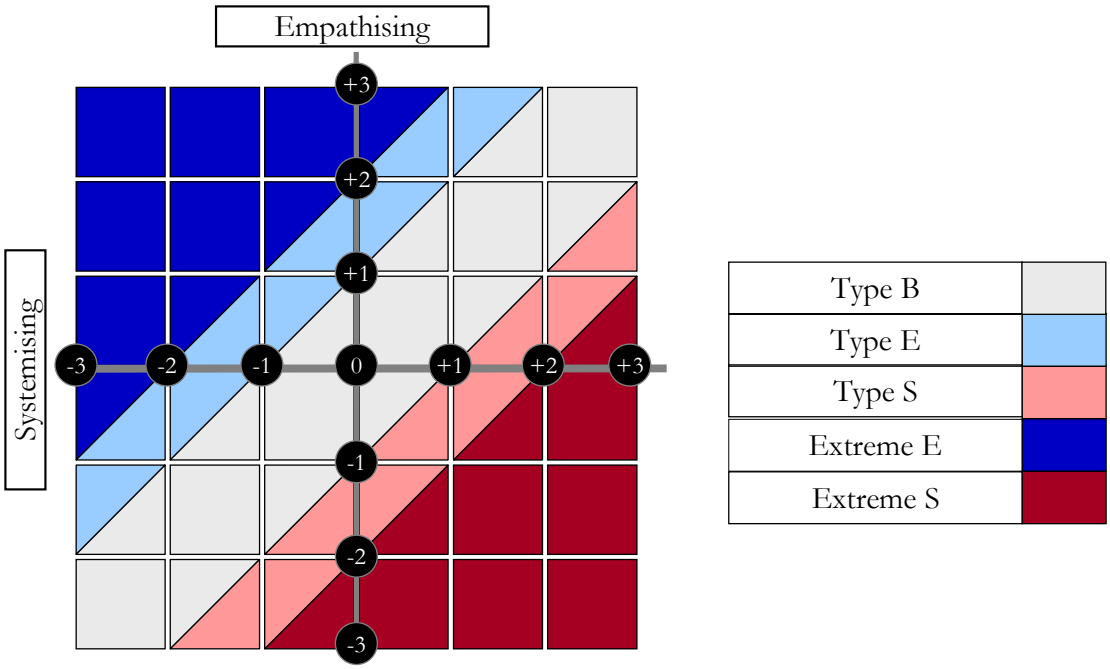
In humans, genetic sex is determined at conception, and there is strong evidence to suggest that ASC are linked to genetic variation (for reviews see Folstein & Rosen-Sheidley, 2001; Gupta & State, 2007; Lauritsen & Ewald, 2001). In particular, ASC have been shown to be strongly heritable, showing concordance rates in monozygotic twins between 60%-95.7% compared to 0%-23.5% of dizygotic twins (Bailey et al., 1995; Ritvo et al., 1985). High concordance rates for a broader spectrum of associated atypical cognitive or social behaviour (or the broader autism phenotype) in monozygotic twins have been estimated at about 90% compared to 10% of dizygotic twins (Bailey et al., 1995). High rates of social and communication difficulties and stereotyped behaviours have also been found in relatives with multiple members who have autism (Bolton et al., 1994; Piven et al., 1997). The expression of many physical and psychological sex differences are also affected by the presence (or absence) of hormones (Goy & McEwen, 1980; Hines, 2004; Kimura, 1999). The relationship between genetic variation, hormone levels and the development of cognitive sex differences are still unknown. As a result, there is no consensus on the mechanism(s) responsible for the male vulnerability to ASC, which is likely to be influenced by genetic, hormonal and environmental effects.

1.2. Empathising and Systemising

The Empathising-Systemising (E-S) theory of typical sex differences proposes that females on average have a stronger drive to empathise (to identify another person's emotions and thoughts, and to respond to these with an appropriate emotion), while males tend to have a stronger drive to systemise (to analyse or construct rule-based

systems, whether mechanical, abstract or another type) (Baron-Cohen, 2002, 2003). The Empathising Quotient (EQ) and Systemising Quotient (SQ) were developed to measure these dimensions in individuals (Baron-Cohen et al., 2003; Baron-Cohen & Wheelwright, 2004). Using the difference between a person's EQ and SQ score, individual cognitive 'brain types' can be calculated where individuals who are equal in their E and S are said to have a balanced (B) brain type (E=S). The Type S (S>E) is more common in males, whilst the Type E (E>S) is more common in females (Goldenfeld, Baron-Cohen & Wheelwright, 2005; Wheelwright et al., 2006). Extreme brain types are also found, and the majority (61.6%) of adults with ASC fall in the Extreme S (S>>E) region, compared to 1% of typical females (Wheelwright et al., 2006). The strong bias towards the 'extreme S' brain type for individuals with ASC gives rise to the 'extreme male brain' (EMB) theory of autism (see Figure 1.1).

Figure 1.1. Brain types



Note: The Empathising-Systemising Model of Typical Sex Differences. The main brain types are illustrated on axes of Empathising (E) and Systemising (S) dimensions (numbers represent standard deviations from the mean). Balanced brain (Type B, grey region); female brain (Type E, light blue region), male brain (Type S, light red region); the extreme Types E (dark blue) and S (dark red) lie at the outer borders. According to the 'extreme male brain' theory of autism, people with ASC will generally fall in the dark red region. Axes show standard deviations from the mean. Figure modified from: Baron-Cohen, S. (2002). The extreme male brain theory of autism. *Trends in Cognitive Sciences*, *6*, 248-254.

1.3. The Extreme Male Brain theory of autism

The EMB theory of autism proposes that ASC are linked to an extreme manifestation of male-typical behaviours. A large body of experimental evidence now exists to support this hypothesis. Some of these findings will be discussed next.

Individuals with ASC score higher on the SQ, a measure on which typical males score higher than typical females (Baron-Cohen et al., 2003; Wheelwright et al., 2006). Individuals with ASC show superior performance compared to controls on the Embedded Figures Test (EFT), a task on which typical males perform better than typical females (Jolliffe & Baron-Cohen, 1997; Shah & Frith, 1983). The EFT requires good attention to detail, assumed to be a prerequisite of systemising. Individuals with ASC have also been found to have either intact or superior functioning on tests of intuitive physics, a domain which shows a sex difference in favour of males in adulthood (Baron-Cohen et al., 2001b; Lawson, Baron-Cohen & Wheelwright, 2004). Sex differences have been found on the Block Design subscale of the WISC-R intelligence test, with typical males performing better than females (Lynn et al., 2005). Children with autism also demonstrate superior functioning on this test (Allen, Lincoln & Kaufman, 1991; Lincoln et al., 1988; Shah & Frith, 1993).

Individuals with ASC show impairment on certain measures where women tend to score higher than men. For example, studies using the EQ report that typical females score higher than typical males, whereas individuals with ASC score lower than controls (Baron-Cohen & Wheelwright, 2004). Individuals with ASC also score lower than control males in the 'Reading the Mind in the Eyes' task (considered to be an advanced test of empathising) (Baron-Cohen et al., 1997), the 'Reading the Mind in the Voice' task (which involves recognition of complex emotions from videos of facial expressions or audios of vocalisations) (Golan, Baron-Cohen & Hill, 2006) and on the Friendship and Relationship Questionnaire (which measures the importance of emotional intimacy and sharing in relationships) (Baron-Cohen & Wheelwright, 2003).

Measures of autistic traits report findings consistent with the EMB theory. For example, the Childhood Autism Spectrum Test (CAST) (Scott et al., 2002b; Williams et al., 2005), (formerly known as the Childhood Asperger Syndrome Test, renamed because it can be used for all subgroups on the autistic spectrum (Baron-Cohen et al., in preparation)) is a parent-report measure developed to screen for ASC in a typical population on which boys score higher than girls (Williams et al., submitted), and children with ASC score higher than typically developing children (Williams et al., 2005). In addition, the Autism Spectrum Quotient (AQ) was developed to help quantify the number of autistic traits an individual displays (Baron-Cohen et al., 2001a). Individuals with Asperger Syndrome or High-Functioning autism score higher on the AQ than those without a diagnosis (Baron-Cohen et al., 2001a). Among controls, males score higher on the AQ than females (Baron-Cohen et al., 2001a). These results are consistent in adults and adolescents (Baron-Cohen et al., 2006a; Baron-Cohen et al., 2001a) as well as cross-culturally (Hoekstra et al., 2008; Wakabayashi et al., 2006; Wakabayashi et al., 2004). Similar results have been found using the Social Responsiveness Scale, a rating scale designed to measure the severity of autistic symptoms, where individuals with an ASC diagnosis score higher than typical males, who in turn score higher than typical females (Constantino & Todd, 2003).

In addition to the evidence at the psychological level, it has been suggested that characteristics of neurodevelopment in autism such as larger overall brain volumes and greater growth of the amygdala during childhood may also represent an exaggeration of typical sex differences in brain development (Baron-Cohen, Knickmeyer & Belmonte, 2005a). Studies using fMRI indicate that typical females show increased activity in the extrastriate cortex during the Embedded Figures Test and increased activity bilaterally in the inferior frontal cortex during the 'Reading the Mind in the Eyes' task. Parents of children with ASC also tend to show hyper-masculinisation of brain activity, suggesting that hyper-masculinisation may be part of the broader autism phenotype (Baron-Cohen et al., 2006b).

It remains important to identify the biological mechanisms that cause such sexual dimorphism. One study has shown sexual dimorphism in looking preferences in n=102 newborn infants who were approximately 37 hours old. Boys were found to exhibit a preference for non-social stimuli (mechanical mobile), whilst girls tended to prefer looking at social stimuli (faces) (Connellan et al., 2000). Although these simple experiments with social versus non-social stimuli are not an indication of ASC, these early sex differences in behaviour suggest a biological basis, since these children have not yet been influenced by social or cultural factors. One possible biological mechanism is the effect of prenatal exposure to hormones, in particular the androgen testosterone (Baron-Cohen, Lutchmaya & Knickmeyer, 2004).

1.4. Hormones and sexual differentiation

Hormones are essential to reproduction, growth and development, maintenance of the internal environment and the production, use and storage of energy (Larsen et al., 2002). There are marked physical and behavioural consequences of exposure to hormones throughout life. Prenatally, the presence or absence of specific hormones (or their receptors) is known to be essential to the sexual differentiation of the foetus. In addition to stimulating development of physical characteristics such as genitalia (Fuchs & Klopper, 1983; Hines, 2004; Kimura, 1999; Novy & Resko, 1981; Tulchinsky & Little, 1994), there is increasing evidence that prenatal hormones have a substantial effect on gender-typical aspects of behaviour (Cohen-Bendahan, van de Beek & Berenbaum, 2005a; Hines, 2004). If this is the case, then the occurrence of these hormones prenatally may have a substantial bearing on the development of an extreme male profile associated with ASC.

1.5. Sex differences in human behaviour

It is anticipated that behaviours showing large sex differences are the best candidates for studying effects of hormones on later development (Cohen-Bendahan et al., 2005a; Collaer & Hines, 1995; Hines, 2004). The use of effect sizes (calculated using 'Cohen's

d') can assist in determining what behaviours may show large sex differences since it provides a standardised measure of the magnitude of group differences that can be compared across varying sample sizes (Cohen, 1988). Table 1.1 shows the representative sample of sex-related behaviours, along with the direction and size of the sex difference in standard deviation units, 'd' (Cohen-Bendahan et al., 2005a).

Table 1.1 Representative sex differences in behaviour

Trait	Direction of sex difference	*d*, Size of sex difference[a]
Cognitive abilities		
Spatial ability: mental rotation	M>F	Large
Spatial ability: targeting	M>F	Large
Verbal ability: fluency	F>M	Small to medium
Verbal ability: memory	F>M	Medium
Perceptual speed and accuracy	F>M	Small to medium
Personality traits		
Sensation-seeking	M>F	Medium to large
Aggression	M>F	Large
Nurturance	F>M	Medium
Interest in babies	F>M	Medium to large
Gender-role behaviours		
Interest in male-typical activities	M>F	Very large
Interest in female-typical activities	F>M	Very large
Preference for boys as playmates	M>F	Very large
Preference for girls as playmates	F>M	Very large
Sexual orientation		
Arousal to females	M>F	Very large
Arousal to males	F>M	Very large

[a] *d* (Mean difference/standard deviation) as reported in adults. Table from: Cohen-Bendahan, C. C., van de Beek, C., & Berenbaum, S. A. (2005). Prenatal sex hormone effects on child and adult sex-typed behavior: Methods and findings. *Neuroscience and Biobehavioral Reviews*, *29*, 353-384.

1.6. Gonadal hormones

The links between hormone levels, physical development and behaviour are complex and not yet fully understood, particularly in terms of effects on early development.

Hormone levels can be measured at particular points in time but levels could vary on a daily basis (van de Beek et al., 2004), and prenatal measurements are very difficult (and potentially dangerous) to obtain for research purposes alone. Furthermore, correlations with behavioural measurements are always complicated by the need to determine the presence of a particular trait, without artificially inducing the behaviour or creating bias in the result. A useful way of controlling some of these factors is to examine results from animals, where it has been possible to directly manipulate and monitor the levels of hormones throughout pregnancy and to control for environmental effects. As a result, researchers often look to confirm effects measured in animals with similar measurements in humans. Even then, the correlation between animals and humans is not always clear cut, with the potential for quite different mechanisms.

Though genetic sex is determined at conception, it is the gonadal hormones (i.e. androgens, oestrogens and progestins (Larsen et al., 2002)) which are responsible for differentiation of the male and female phenotypes in the developing human foetus (Fuchs & Klopper, 1983; Hines, 2004; Kimura, 1999; Novy & Resko, 1981; Tulchinsky & Little, 1994). Androgens such as testosterone are of particular interest to the study of male-typical behaviour because when these androgens and the appropriate receptors are present, the male genital phenotype will develop. If androgens (or their receptors) are not present, then the female genital phenotype will develop (such as in female foetuses or genetic males with Complete Androgen Insensitivity Syndrome) (George & Wilson, 1992; Jost, 1961, 1970, 1972). Another hormone which forms from prenatal testosterone is the oestrogen hormone oestradiol, which has been observed to promote male-typical behaviour in rats and other rodents (Collaer & Hines, 1995). In humans, the relative contribution of oestradiol to development of male-typical behaviours is less certain, since studies have not shown significant associations with the development of later behaviour (Knickmeyer et al., 2005a; Knickmeyer et al., 2006b; Knickmeyer et al., 2005b; Lutchmaya, Baron-Cohen & Raggatt, 2002a; Lutchmaya, Baron-Cohen & Raggatt, 2002b; van de Beek et al., 2004; van de Beek et al., 2008).

Behavioural studies in nonhuman mammals have shown that the same prenatal hormones that are involved in sexual differentiation of the body are also involved in sexual differentiation of behaviour (Breedlove, 1992; Goy & McEwen, 1980). In animals, higher doses of hormones have been seen to masculinise behaviour more than lower doses, though the effect of concentration is not uniform for different behaviours (Goy & McEwen, 1980). Effects are also likely to be non-linear and include both lower and upper threshold values, beyond which changes in concentration have no effect (Cohen-Bendahan et al., 2005a). The interaction between hormones may also be important (Goy & McEwen, 1980).

1.7. Atypical foetal hormone environments

In humans, the manipulation or even direct measurement of hormone levels in healthy humans is considered unethical because of the potential dangers involved. However, some studies have investigated abnormal hormone environments which lead to particular medical conditions. Such conditions can lead to considerable difficulties for the individual and are fortunately rare. However, some studies have obtained sufficient participation to render useful information about how abnormal environments influence behaviour. A detailed review of many of the studies surrounding these conditions has been provided elsewhere (Baron-Cohen et al., 2004; Cohen-Bendahan et al., 2005a; Hines, 2004; Knickmeyer & Baron-Cohen, 2006b), so this discussion will focus on findings relevant to characteristics of ASC.

1.7.1. *Congenital Adrenal Hyperplasia*

Congenital Adrenal Hyperplasia (CAH) is a genetic disorder affecting both sexes which causes excess adrenal androgen production beginning prenatally (New, 1998). CAH affects both males and females but is most clearly observed in females because of their typically low androgen levels. Female foetuses with CAH have similar androgen levels to those found in typical males (Hines, 2004). Behavioural studies of females with CAH

show a more masculinised profile compared to unaffected female siblings or matched controls.

In terms of specific behaviours, girls with CAH show masculinisation of characteristics typically associated with males. These include spatial orientation, visualisation, targeting, personality, cognitive abilities and sexuality (Hampson, Rovet & Altmann, 1998; Hines et al., 2003b; Resnick et al., 1986). Females with CAH are also likely to be more interested in male-typical activities and less interested in female-typical activities throughout life (Berenbaum, 1999; Berenbaum & Hines, 1992; Berenbaum & Snyder, 1995; Ehrhardt & Baker, 1974; Hines, Brook & Conway, 2004).

Studies relating CAH and autism are limited. Since the condition is typically associated with masculinisation, effects are more apparent in girls than boys. Results from one study of girls with CAH suggests that they exhibit more autistic traits, measured by the AQ, compared to unaffected females (Knickmeyer et al., 2006a). Individuals with CAH also demonstrate higher levels of language and learning difficulties compared to unaffected family members (Resnick et al., 1986).

Whilst CAH provides an interesting window on additional androgen exposure, the relatively rare occurrence of CAH in conjunction with ASC makes it difficult to obtain large enough sample sizes for generalisation to the wider population. In addition, some researchers have suggested that CAH-related disease characteristics, rather than prenatal androgen exposure, could be responsible for the atypical cognitive profiles observed in this population (Fausto-Sterling, 1992; Quadagno, Briscoe & Quadagno, 1977).

1.7.2. *Complete Androgen Insensitivity Syndrome*

Complete Androgen Insensitivity Syndrome (CAIS) occurs when there is a complete deficiency of androgen receptors and is more common in males, with incidence between 1 in 60,000 and 1 in 20,000 births. At birth, genetic male infants with CAIS are

phenotypically female despite an XY (male typical) complement and are usually raised as girls with no knowledge of the underlying disorder. Although breasts develop, diagnosis usually takes place when menarche fails to occur (Larsen et al., 2002; Nordenstrom et al., 2002).

The investigation of behaviours (such as gender identity, sexual orientation, gender role behaviour in childhood and adulthood) and personality traits that show sex differences have suggested that males with this condition do not significantly differ from same-sex controls (Hines, Ahmed & Hughes, 2003a; Quadagno et al., 1977). In addition, and hand preferences have also been shown to not differ between these individuals and same-sex controls (Hines et al., 2003a). However, other results suggest that individuals with CAIS tend to show feminised performance on tests of visuo-spatial ability (Money, Schwartz & Lewis, 1984). If replicable, this finding lends support to the notion that androgens enhance male-typical behaviours. Specific evidence for ASC is not available due to the rare incidence of this condition.

1.7.3. *Idiopathic Hypogonadotrophic Hypogonadism (IHH)*

Idiopathic Hypogonadotrophic Hypogonadism (IHH) occurs when an individual's gonads lack sufficient stimulation to produce normal levels of hormones. This can occur congenitally or after puberty. These individuals have normal male genitalia at birth, so it can be assumed that their prenatal testosterone levels were normal (Knickmeyer & Baron-Cohen, 2006b). Men with IHH perform worse on the Embedded Figures Test, the Space Relations and Block Design subtests of the Weschler Adult Intelligence Scale, when compared with normal males and males with acquired hypogonadotrophic hypogonadism after puberty (Hier & Crowley, 1982). However, another study found that males with IHH do not show deficits on similar scales of intelligence (Cappa et al., 1988). More research needs to be conducted in order to resolve these findings and relate the effects to ASC.

1.8. Hormonal effects: Indirect studies in typical populations

There is a steady body of evidence which indicates that foetal hormone levels influence certain physical characteristics which can be observed after birth. These 'proxy' measurements have been used to indicate levels of prenatal androgen exposure and have been examined extensively in relation to behavioural traits. Several reviews of these measurements exist (see Cohen-Bendahan et al., 2005a; Kimura, 1999) and this discussion will be focused on studies related to behaviours associated with ASC.

1.8.1. *Digit Ratio (2D:4D)*

The ratio between the length of the 2nd and 4th digit (2D:4D) of the hand has been found to be sexually dimorphic, being lower in males than in females. 2D:4D ratio is thought to be fixed by week 14 of foetal life and it has been hypothesised that it might reflect foetal exposure to prenatal sex hormones in early gestation (Manning, 2002).

Measurements indicate an association between the ratio of FT and foetal oestradiol levels and 2D:4D ratio for the right hand after controlling for sex (Lutchmaya et al., 2004). For subjects with CAH, females show lower (more masculinised) 2D:4D on the right hand compared to unaffected females, and men with CAH have lower 2D:4D on the left hand compared to unaffected males (Brown et al., 2002). Results in this sample are consistent with the notion that prenatal androgen exposure masculinises 2D:4D ratio. This measure has been widely used as a proxy for prenatal testosterone exposure due to the ease and simplicity of measurement. However, it is likely that 2D:4D ratio is affected by multiple factors (Cohen-Bendahan et al., 2005a).

Findings in studies with 2D:4D ratios tend to support the suggestion that higher FT levels are a risk factor for ASC. Lower (i.e. hyper-masculinised) digit ratios have been found in children with autism compared to typically developing children, and this was also found in the siblings and parents of children with autism, indicating a genetic basis for elevated FT levels in autism (Manning et al., 2001; Milne et al., 2006).

1.8.2. *Dermatoglyphics*

Dermatoglyphics, or fingerprints, have also been used as a proxy measure for prenatal exposure to testosterone. The number of dermal ridges is thought to be fixed by about the fourth month of gestation (Holt, 1968). Researchers have focused on total finger ridge count and asymmetry between left and right hands. Sex differences have been observed in ridge count with males exhibiting more ridges in total than females. Both sexes typically have more ridges on the right hand than on the left hand (R>L). Sex differences have also been observed in asymmetry, and the left greater than right (L>R) pattern is more common in females than in males (Kimura, 1999).

Studies examining total ridge count in adults and children have shown that for both men and women who exhibit the L>R pattern, performance was better for tasks that show a female superiority such as verbal fluency and perceptual speed (Kimura & Carson, 1995; Kimura & Clarke, 2001; Sanders & Waters, 2001). The opposite pattern was found for those exhibiting the R>L pattern, who demonstrated better performance for tasks that show a male superiority (Kimura & Carson, 1995; Kimura & Clarke, 2001; Sanders & Waters, 2001).

Data from dermatoglyphic patterns and their relation to autism are limited and conflicting. In one study 78 children with autism were compared to the same number of matched controls (Walker, 1977). Analysis of ridge patterns and ridge counts resulted in significant differences between the children with and without autism. Children with autism typically exhibited lower ridge count and less distinct fingerprint features (Walker, 1977). However, a smaller study comparing children with autism, learning difficulties and typical children found no significant differences for ridge counts (Hartin & Barry, 1979).

As with the 2D:4D ratio, studies using dermatoglyphics may be useful, but more evidence is needed to establish whether there is a link between dermatoglyphics and

prenatal hormone exposure. In addition, further studies are needed to understand the potential links with ASC. The few studies of dermatoglyphics in ASC are quite dated, and in more recent decades clinicians and researchers have become more alert to detecting autism in higher-functioning individuals (such as those with Asperger syndrome) and it would be of interest to repeat these early studies with the range of subgroups on the autistic spectrum.

1.8.3. *Lateralisation*

It has been proposed that some observable sex differences in human behaviour and cognition may be accounted for by differences in cerebral lateralisation (Hines & Shipley, 1984). In addition to research investigating functional asymmetries in the brain, body asymmetries (other than fingerprint asymmetries) have been associated with prenatal sex hormones (Kimura, 1999).

Levels of FT have been implicated in left-handedness and asymmetrical lateralisation (Fein et al., 1985; McManus et al., 1992; Satz et al., 1985; Soper et al., 1986). Left-handedness and ambidexterity are more common in typical males (Peters, 1991) as well as in individuals with CAH (Nass et al., 1987) and autism (Gillberg, 1983). In addition, the typical male brain is heavier than the female brain (Harden et al., 2001), a difference that may in part be due to early FT exposure (Hines, 2004).

1.8.4. *Pubertal onset*

Pubertal onset has been used to investigate variations in hormones. Females typically enter puberty earlier than males (Cohen-Bendahan et al., 2005a). Research examining the physical indicators of hormone exposure and autism have found that a subset of male adolescents with autism show hyper-androgeny, or elevated levels of androgens, and precocious puberty (Tordjman et al., 1997). These findings suggest that individuals with autism have atypical hormonal activity around the time of puberty. Other research has also shown that androgen-related medical conditions such as polycystic ovary

syndrome (PCOS), ovarian growths, and hirsutism occur with elevated rates in both women with Asperger syndrome and in mothers of children with autism (Ingudomnukul et al., 2007). Delayed menarche has also been observed in females with Asperger syndrome (Ingudomnukul et al., 2007; Knickmeyer et al., 2006c).

1.8.5. *Co-twin sex*

Nonhuman studies examining the effects of animal position in the uterus have suggested that the sex of littermates can affect the development of sex-typical behaviours (Clark & Galef, 1998). For rodents, masculinisation of females was seen to occur when they were positioned between two males in the uterus. For multiple littermates, the blood supply is channelled between foetuses and in another study it was found that females developed more male-typical traits if they were 'downstream' of their male littermates (Hines, 2004).

For human twins, it is thought that females adjacent to a male will demonstrate masculinised behaviour as a result of testosterone transfer from the male (Even, Dhar & vom Saal, 1992; Fels & Bosch, 1971; Meisel & Ward, 1981). There is also some evidence that human males with an opposite-sex twin exhibit feminised gender-role behaviour (Elizabeth & Green, 1984). However, most studies have not observed feminisation (Cole-Harding, Morstad & Wilson, 1988; Elkadi, Nicholls & Clode, 1999; Miller & Martin, 1995; Resnick, Gottesman & McGue, 1993; Rodgers, Fagot & Winebarger, 1998a). Other investigations of gender-typical play have also failed to find opposite-sex twin effects (Henderson & Berenbaum, 1997; Rodgers et al., 1998a). Moreover, differences in human twins are difficult to interpret because they may result from the social effects of having an opposite-sex twin, rather than an effect of hormonal exposure during gestation (Cohen-Bendahan et al., 2005a).

It is widely accepted that genes play a role in the aetiology of autism (Bailey et al., 1995; Folstein & Rosen-Sheidley, 2001; Gupta & State, 2007; Lauritsen & Ewald, 2001). In the

absence of any known gene or genes, the main support for this is derived from family and twin studies. Two recent studies suggest that the twinning process itself may be an important risk factor in the development of autism (Betancur, Leboyer & Gillberg, 2002; Greenberg et al., 2001). Both studies compared the number of twin pairs among affected sibling pairs to expected values. Results showed a significant excess of twin pairs. However, data from other studies do not support twinning as a substantial risk factor in the aetiology of autism (Croen, Grether & Selvin, 2002; Hallmayer et al., 2002; Hultman, Sparen & Cnattingius, 2002). The high proportion of twins found in affected sibling pair studies could be explained by the high concordance rates in monozygotic (MZ) twins versus siblings (Hallmayer et al., 2002).

Researchers have suggested that environmental factors associated with various demographic characteristics such as sex, multiple births, maternal age and education may interact with genetic vulnerability to increase the risk of autism (Croen et al., 2002). However, no firm conclusions can be drawn at the present time.

1.9. Hormonal effects: Direct studies of hormone effects

Since differences in sex-typical behaviour can be observed shortly after birth, prenatal exposure to hormones may be an important candidate. Various measures have been identified to help examine the effects of prenatal hormones on later development. Whilst some of these measures appear to offer support for the EMB theory, analysis of research findings in section 1.8 highlights the inconsistencies encountered when using proxy measures to investigate the effect of hormones on behaviour.

At present there is little direct support for these predictors as a way of studying prenatal hormone influence. The ideal study of the effect of hormones on later development would involve a series of direct measurements at regular intervals throughout gestation and into postnatal life. In practice these measurements are complicated by effects of timing and accessibility.

1.10. Timing effects

The timing of hormonal effects is crucial when studying lasting effects on development. There are thought to be two general types of hormonal effects: organisational and activational (Phoenix et al., 1959). Organisational effects are most likely to occur during early development when most neural structures are established, producing permanent changes in the brain (Phoenix et al., 1959), whereas activational effects are short term and are dependent on current hormone levels. Since ASC are typically persistent with an early onset, any hormonal influence on the development of ASC is likely to be organisational in nature.

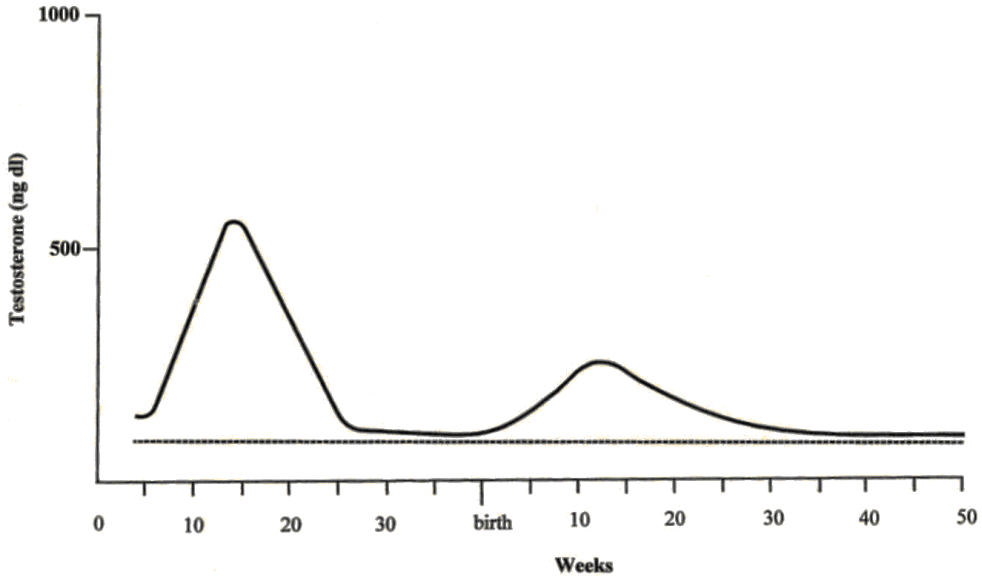
It is widely thought that organisational effects are maximal during sensitive periods. These are hypothetical windows of time in which a tissue can be formed (Hines, 2004). Outside the sensitive period, the effect of the hormone will be limited, protecting the animal from disruptive influences. This means, for example, that circulating sex hormones necessary for adult sexual functioning do not cause unwanted alterations to tissues, even though the same hormones might have been essential to the initial development of those tissues. The importance of sensitive periods for behavioural development was seen by Goy et al. (1988), who found that androgens masculinise different behaviours at different times during gestation in rhesus macaques.

For typical human males, there is believed to be a surge in FT at around weeks 8-24 of gestation (Baron-Cohen et al., 2004; Collaer & Hines, 1995; Hines, 2004; Smail et al., 1981), with a decline to barely detectable levels from the end of this period until birth. As a result, any effects of FT on development are most likely to be determined during this period. For typical human females, levels are generally very low throughout pregnancy and childhood (Hines, 2004).

In addition to the foetal surge, two other periods of elevated testosterone have been observed in typical males. The first takes place shortly after birth and lasts for

approximately 3-4 months (Smail et al., 1981), after which levels return to very low levels until puberty. Figure 1.2 shows the circulating levels of testosterone during the prenatal and neonatal period. Research has shown that neonatal testosterone is important for genital development (Brown et al., 1999), but the evidence for its role in behavioural development is unclear. Few studies have been conducted on the effects of neonatal testosterone.

Figure 1.2. Prenatal and neonatal circulating testosterone levels



Circulating levels of testosterone in the human foetus and neonate. Males (solid line) have higher levels of testosterone than females (dashed line), particularly from about weeks 8-24 of gestation and weeks 2-26 of postnatal life. Figure from: Hines, M. (2004). *Brain gender*. New York, New York: Oxford University Press, Inc.

In later life, early pubertal effects are the first visible indications of rising androgen levels in childhood, and occur in both boys and girls. Due to the early onset of ASC, the pubertal surge in testosterone is of less interest in determining the aetiology of these conditions. However, there is an increasing body of evidence which suggests that prenatal androgens may be involved in determining sexually dimorphic traits. In the remainder of this chapter, direct measurements of testosterone and associations with the development of ASC are discussed.

1.10.1. *Maternal sampling during pregnancy*

Various studies have measured testosterone levels in maternal blood during pregnancy (Hines et al., 2002a; Udry, 2000; Udry, Morris & Kovenock, 1995). One study found that androgen exposure in the second trimester was positively associated with male-typical behaviour in adult females (Udry et al., 1995). Similar findings in another study revealed that higher levels of testosterone in mothers were associated with masculinised gender-role behaviour in 3.5 year old girls, but not boys (Hines et al., 2002a). These findings support the suggestion that higher FT levels masculinise behaviour. No study to date has used maternal testosterone levels to investigate the development of autistic traits. However, samples of maternal testosterone may not reflect the foetal levels, since the foetus is thought to be protected from maternal hormones by the placenta (Cohen-Bendahan et al., 2005a). In order to examine the effects of foetal hormonal exposure, more direct measurements are desirable.

1.10.2. *Samples from the umbilical cord*

A series of studies have examined relationships between umbilical cord (perinatal) hormones and later behaviour such as temperament and mood. Some studies report that high perinatal testosterone and oestradiol levels were significantly related to low timidity in boys but not girls (Jacklin, Maccoby & Doering, 1983; Jacklin, Wilcox & Maccoby, 1988; Marcus et al., 1985). Other studies of umbilical cord hormones have shown inconsistent results (Abramovich & Rowe, 1973; Forest et al., 1974; Pang et al., 1979).

Levels of FT are typically at very low levels from about week 24 of gestation, whereas the neonatal peak has not yet appeared. In addition, the umbilical cord contains blood from the mother as well as the foetus, and hormone levels may vary due to labour itself (Jacklin et al., 1988). These factors are believed to contribute to the inconsistencies observed in studies using umbilical cord samples.

1.10.3. *Amniotic Fluid*

One of the most promising methods for obtaining information about foetal exposure to androgens appears to be the direct sampling of FT levels in amniotic fluid, obtained from routine diagnostic amniocentesis. This is performed for clinical reasons in order to detect genetic abnormalities in the foetus. As a result, it is typically performed during a relatively narrow time period which is thought to coincide with the peak in foetal testosterone for male foetuses. This peak is also apparent in amniotic fluid and several studies have documented a large sex difference in amniotic androgens (Dawood & Saxena, 1977; Finegan, Bartleman & Wong, 1989; Judd et al., 1976; Nagamani et al., 1979; Robinson et al., 1977). There are significant risks associated with the procedure itself, so that it cannot be performed solely for research. However, the process itself does not appear to have any negative effects on later development (Judd et al., 1976).

The origins of androgens in amniotic fluid are not fully understood, but the main source seems to be the foetus itself (Cohen-Bendahan et al., 2005a). Hormones enter the amniotic fluid in two ways: via diffusion through the foetal skin in early pregnancy, and via foetal urine in later pregnancy (Judd et al., 1976; Schindler, 1982). Given the risk involved in obtaining blood from the foetus, there are very limited data directly comparing testosterone in amniotic fluid to that in foetal blood. Androgens in amniotic fluid are unrelated to androgens measured in maternal blood in the same period, as shown in studies in early and mid-gestation (Rodeck et al., 1985; van de Beek et al., 2004). Based on these findings, testosterone obtained in amniotic fluid appears to be a good reflection of the levels in the foetus, and represents an alternative to the more risky process of collecting foetal serum (Cohen-Bendahan et al., 2005a).

Finegan et al. (1992) conducted the first study which explored the relationship between prenatal hormone levels in amniotic fluid and later behaviour on a broad range of cognitive functions in 4-year-old children. The findings are difficult to interpret since the authors used measures that did not show sex differences. However, the same

children were followed up at 7 years of age and associations between spatial ability and FT were examined (Grimshaw, Sitarenios & Finegan, 1995b). A significant positive association between FT levels and faster performance on a mental rotation task was observed in a small subgroup of girls, but not boys. At 10 years of age, prenatal testosterone levels were found to relate to handedness and dichotic listening tasks (Grimshaw, Bryden & Finegan, 1995a), and the results were interpreted as providing support for the hypothesis that higher levels of prenatal sex hormones are related to lateralisation in boys and girls (Witelson, 1991).

1.10.4. *Cambridge Foetal Testosterone Project*

The Cambridge Foetal Testosterone Project is an ongoing longitudinal study investigating the relationship between FT levels and the development of behaviours relating to ASC (Baron-Cohen et al., 2004; Knickmeyer & Baron-Cohen, 2006a). Mothers of participating children had all undergone amniocentesis for clinical reasons between 1996 and 2001 and gave birth to healthy singleton infants. To date, these children have been tested postnatally at 12 months, 18 months, 24 months, 4 years and 5 years of age.

1.10.4.1. FT and eye contact at 12 months

Reduced eye contact is a characteristic common in children with autism (Lutchmaya et al., 2002a; Swettenham et al., 1998). The first study aimed to measure FT and oestradiol levels in relation to eye contact for a sample of 70 typically developing, 12-month old children (Lutchmaya et al., 2002a). Frequency and duration of eye contact were measured using videotaped sessions. Sex differences were found, with girls making significantly more eye contact than boys. The amount of eye contact varied quadratically with FT levels when the sexes were combined. Within the sexes, a relationship was only found for boys (Lutchmaya et al., 2002a). No relationships were observed between the outcome and oestradiol levels. Results were taken to indicate that FT may play a role in

shaping the neural mechanisms underlying social development (Lutchmaya et al., 2002a).

1.10.4.2. FT and vocabulary at 18 and 24 months

In some subgroups within ASC, such as classic autism, vocabulary development is also delayed (Rutter, 1978). Another study (of 87 children) focused on the relationship between vocabulary size in relation to FT and oestradiol levels from amniocentesis. Vocabulary size was measured using the Communicative Development Inventory, which is a self-administered checklist of words for parents to complete (Hamilton, Plunkett & Shafer, 2000). Girls were found to have significantly larger vocabularies than boys at both time points (Lutchmaya et al., 2002b). Results showed that levels of FT inversely predicted the rate of vocabulary development in typically developing children between the ages of 18 and 24 months (Lutchmaya et al., 2002b). Within sex analyses showed no significant relationships in boys or girls, which the authors believe may have been due to the relatively small sample sizes. No relationships between oestradiol and vocabulary size were found. Despite the lack of significant results within sex, the significant findings in the combined sample suggest that FT may be involved in shaping the neural mechanisms underlying communicative development (Lutchmaya et al., 2002b).

1.10.4.3. FT and empathy at age 4

Thirty-eight children completed a 'moving geometric shapes' task at age 4 where they were asked to describe cartoons with two moving triangles whose interaction with each other suggested social relationships and psychological motivations (Knickmeyer et al., 2006b). Sex differences were observed with girls using more mental and affective state terms to describe the cartoons compared to boys, however no relationships between FT levels and mental or affective state terms were observed. Girls were found to use more intentional propositions than males, and a negative relationship between FT levels and frequency of intentional propositions was observed when the sexes were combined and

in boys. Boys used more neutral propositions than females, and FT was related to the frequency of neutral propositions when the sexes were combined. However, no significant relationships were observed when boys and girls were examined separately. No relationships with oestradiol were observed. These results are consistent with the EMB theory since other studies have found that individuals with ASC perform lower than typical males on a similar moving geometric shapes task (Klin, 2000).

1.10.4.4. FT, restricted interests and social relationships at age 4

Individuals with ASC demonstrate more restricted interests as well as difficulties with social relationships (APA, 1994). A second follow-up at 4 years of age in this same cohort of children utilised the Children's Communication Checklist (Bishop, 1998). The quality of social relationships subscale demonstrated an association between higher FT levels and poorer quality of social relationships for both sexes combined but not individually. A lack of findings in within sex analyses was thought to be a result of the small sample size (n=58).

Levels of FT were also associated with more narrow interests when the sexes were combined and in boys only (Knickmeyer et al., 2005a). Sex differences were reported, with males scoring higher (i.e. having more narrow interests) than females (Knickmeyer et al., 2005a).

1.10.4.5. FT and gender-typed play at age 5

At 5 years of age, the mothers of children were asked to complete a modified version of the Child Game Participation Questionnaire (Bates & Bentler, 1973). No significant relationship between levels of FT and game participation were observed when the entire group was included in the analysis, or when boys and girls were examined separately. These findings may reflect a relatively small sample size (n=53) or perhaps an insufficiently sensitive behavioural measure. However, findings in this study suggest that

hormonal influences on behaviour are complex, and issues such as hormonal timing and dosage need to be considered.

1.11. Objectives

Existing results from the Cambridge FT longitudinal study suggest a link between early development of empathising behaviours and lower exposure to FT. Whilst these measurements did not specifically investigate associations in individuals with an ASC, these behaviours are characteristic of individuals with a clinical diagnosis. Baron-Cohen (2002) suggests that apart from genetic influences, the effects of prenatal testosterone on the brain may be a candidate biological mechanism responsible for the extreme male pattern observed in certain sexually dimorphic traits.

Although ASC is considered to be a strongly genetic condition, a wide range of indirect and direct evidence points to gonadal hormones (in particular foetal testosterone) affecting the development of behaviours associated with ASC. The purpose of the studies presented in the following chapters is to examine this link in more detail by investigating aspects of child development that have shown sexual dimorphism and/or atypical performance patterns in individuals with ASC, and their relationship to FT levels (measured in amniotic fluid obtained during routine amniocentesis). In particular, this thesis tests whether the effects of FT are broad (i.e. showing a relationship between all male-typical traits, such as spatial ability and aggression) or specific (i.e. showing a relationship between specific traits relating to empathy, systemising, attention to detail, and autistic traits).

Chapters 2, 3 and 4 will investigate behaviours that show large sex differences, namely spatial ability, gender-role behaviour and aggression and possible associations with FT levels. Chapters 5 and 6 examine the E-S and EMB theories in children as well as the relationship between these domains and FT. Chapter 7 is a further test of the relationship between FT and autistic traits in toddlers. The relationship between

prenatal and neonatal testosterone levels will also be examined. Chapter 8 provides a summary of the results and a general discussion will follow.

1.12. Cambridge FT Project Participants

The Cambridge FT Project encompasses participants from two different birth cohorts. Birth Cohort 1 was a pre-existing database of women whose children range between the ages 6-10 years. In Birth Cohort 1, medical records of approximately 950 patients who had undergone amniocentesis in the Cambridge region between June 1996 and June 1997 were examined.

Birth Cohort 2 included mothers who were asked for consent to participate in research at the time of having an amniocentesis (using the new Addenbrooke's Hospital consent form 2004+). The medical records of approximately 700 patients were examined, who had undergone amniocentesis in the Cambridge region between January 2004 and July 2006.

For both birth cohorts, participants were excluded if: (a) amniocentesis revealed a chromosomal abnormality; (b) the pregnancy ended in miscarriage or termination; (c) the child suffered neonatal or infant death; (d) the child suffered significant medical problems after birth; (e) there was a twin pregnancy or (f) the relevant information was absent from medical records. Questionnaires were sent to all mothers whose General Practitioner gave consent. Sample sizes vary since mothers have been participating over a long period of time, and families are not always able to participate in all studies, and/or questionnaires from mothers who do not wish to participate are not returned. The study had full ethical approval from the West Suffolk Multiregional Ethics Committee (April 2005).

Chapters 2 to 6 included participants from Birth Cohort 1. Chapter 7 included participants from Birth Cohort 2. Level of foetal testosterone, measured in amniotic

fluid obtained during routine amniocentesis was the predictor variable of greatest interest. A range of sociodemographic variables were also included in the current series of studies including maternal age, level of education obtained by the parents, presence of older siblings and child age. The sample from the Cambridge FT Project was predominantly Caucasian. As a result, ethnicity was not included in these studies as a predictor variable.

Chapter 2: Foetal testosterone and spatial ability

Much of what is known about sex differences comes from studies of cognition. This chapter investigates whether FT levels measured in second trimester amniotic fluid are related to performance in a series of cognitive tasks that have shown sex differences in adults: Mental Rotation, Embedded Figures Test and Targeting. Intelligence Quotient (IQ) data were also collected. Sample sizes varied from n=74 to n=100 children for each measure. Results showed that FT is positively related to Embedded Figures scores in both boys and girls. This measure also demonstrated sex differences, with boys scoring higher than girls. No significant sex differences were observed for IQ, Mental Rotation or in Targeting. In addition, no significant relationships were observed between FT and IQ, Mental Rotation or Targeting. The current findings provide some support for the EMB theory of autism.

2.1. Introduction

Much of what is known about psychological sex differences comes from studies of cognition. Research has shown that in general, men are better at spatial and non-verbal tasks, whereas women are better at verbal and social tasks (Kimura, 1999). Several examples illustrate this idea. Males demonstrate an advantage in targeting tasks, and females demonstrate an advantage in fine motor tasks (Hall & Kimura, 1995). Males tend to use spatial cues when navigating, and women rely more on landmarks and also have a superior memory for object location (Kimura, 1999). Females are also superior in verbal abilities, and tend to have better verbal memory, spelling ability and verbal fluency in adulthood (Kimura, 1999). However, they have not been found to have larger vocabularies than males. Studies in children have reported greater vocabularies and faster rates of language acquisition in girls (Fenson et al., 1994; Hyde & Linn, 1988).

The largest sex differences in human adult cognitive performance have been observed in certain visuospatial abilities, particularly in mental rotation (the ability to rotate figures quickly and accurately in the mind). Sex differences have also been observed in children, with an increase in the magnitude of sex differences in performance with age (Voyer, Voyer & Bryden, 1995). Due to the large sex differences in cognitive performance, various studies have examined the possibility that exposure to hormones in the prenatal environment may affect these abilities.

Direct studies of prenatal hormone exposure and mental rotation have shown that girls with higher levels of FT (measured in amniotic fluid obtained during amniocentesis) performed a mental rotation task faster than girls with lower FT levels at age 8 (Grimshaw et al., 1995b). Results from this study, however, need to be interpreted with caution, since this finding was only significant in girls who used a rotational strategy (n=12). Although these relations were not observed in boys, the authors suggest that results in girls indicate an effect of testosterone on the foetal brain (Grimshaw et al., 1995b).

An investigation of a link between the hormone proxy measure of digit (2D:4D) ratio and enhanced spatial ability demonstrated a significant association (Manning & Taylor, 2001). Other results, however, suggest digit ratio does not predict mental rotation (Falter, Arroyo & Davis, 2006; Hampson, Ellis & Tenk, 2008). Other studies of prenatal hormonal effects have also produced inconsistent results. One study of cognitive functioning in girls with Congenital Adrenal Hyperplasia (CAH) has shown enhanced mental rotation ability (Resnick et al., 1986), whereas a more recent study did not (Hines et al., 2003b). Other research indirectly examining the effects of hormones on behaviour have shown that human females with male co-twins show masculinised patterns of mental rotation performance (Cole-Harding et al., 1988).

Targeting performance is another area where large sex differences have been observed. Targeting is the ability to aim projectiles accurately at a specified point in space, utilising aspects of spatial skills and motor performance (Hines et al., 2003b). Evidence implicating prenatal hormonal effects has been found for digit ratio, which predicts performance on a computerised targeting task in both children and adults (Falter et al., 2006; Falter, Plaisted & Davis, 2008). Females with CAH have also shown better targeting performance than unaffected females, resembling males with CAH and unaffected males in their performance (Hines et al., 2003b).

Similar experiments have been conducted using the Embedded Figures Test (EFT), where the subject is shown a complex design and asked to find a simple shape within the complex design (Shah & Frith, 1983). Research investigating differences in performance on the EFT has found modest sex differences (Kimura, 1999). In children, boys are quicker and more accurate in locating the target embedded within the larger, complex pattern (Nebot, 1988). Falter et al. (2006) also conducted a study in adults, investigating the relationships between performance on two EFT tasks and digit ratio. Results revealed a significant linear relationship between EFT and digit ratio for one task, but the second task showed no relationships with digit ratio. Another study found superior EFT performance in children with ASC, but EFT scores were not related to

digit ratio (Falter et al., 2008). Other studies where children with autism have completed the EFT obtained results showing that they perform at a level above that of their general mental age (Shah & Frith, 1983). In a study comparing adults with and without ASC, adults with ASC also showed superior performance (Jolliffe & Baron-Cohen, 1997).

Investigations of general cognitive ability or intelligence (IQ) have generally shown negligible sex differences (Halpern, 1997; Hines, 2004; Kimura, 1999). However, some subtests of these standardised measures have shown sex differences, with a female advantage for Digit symbol/Coding and a male advantage on Information and Block Design scales (Hines, 2004). The sex difference observed in the Block Design subscale of the Weschler scales is of particular interest since this is also a task on which individuals with ASC have been shown to excel (Allen et al., 1991; Lincoln et al., 1988; Shah & Frith, 1993).

2.1.1. *Aims*

The evidence outlined above generally points towards a link between prenatal hormone exposure and spatial ability. The remainder of this chapter examines this possibility in more detail by comparing direct measures of FT levels with performance on tasks that have shown sex differences: Mental Rotation, Targeting and Embedded Figures. The relationship between intelligence (IQ) and FT levels is also explored, in order to examine whether FT is correlated with general cognitive ability. Finally, scores on the Block Design component of the Wechsler Abbreviated Scale of Intelligence (WASI) are investigated, because this is also a task on which individuals with autism show enhanced performance (Allen et al., 1991; Lincoln et al., 1988; Shah & Frith, 1993).

2.2. Methods

2.2.1. *Participants*

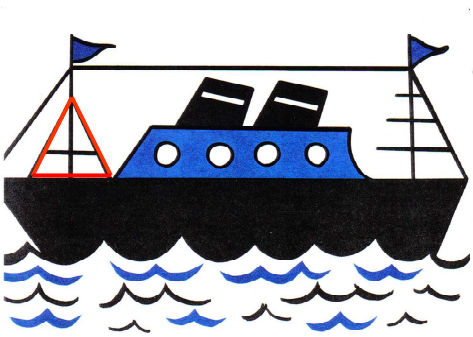
The children in this study were recruited from Birth Cohort 1 of the Cambridge FT Project. This follow-up of children from the Cambridge FT Project was focused on administering tasks that have shown sex differences in adults and to examine which, if any, of these tasks are associated with FT levels. Families were invited to come to Cambridge for cognitive testing resulting in n=100 children (50 boys, 50 girls) taking part (n=456 mothers contacted). Due to the large number and time consuming nature of the battery of tests, not all tests were administered to all participants. As a result, sample sizes vary across outcome variables.

2.2.2. *Outcome variables*

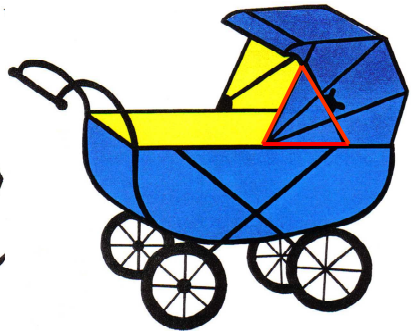
The Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). This scale was used to measure Intelligence Quotient (IQ). The WASI provides scores for Verbal IQ, Performance IQ and Full Scale IQ. The relationship between FT and the Block Design component of the WASI is also examined.

Children's Embedded Figures Test (EFT) (Witkin et al., 1971). This test is administered to children ages 5-12 years. It is designed to assess field dependence and/or independence. This task requires the child to find a simple 'tent' or 'house' shape in progressively more complex drawings and then to trace the shape to indicate where they see it. The 'tent' series is administered before the house series and includes four demonstration items, two practice items and ten scored items. If the child completes the 'tent' series, then the 'house' series is administered and includes four demonstration items, one practice item and 14 scored items. The total score represents the number of figures correctly located (maximum score of 24). Testing is discontinued after five consecutive incorrect responses. See Figure 2.1.

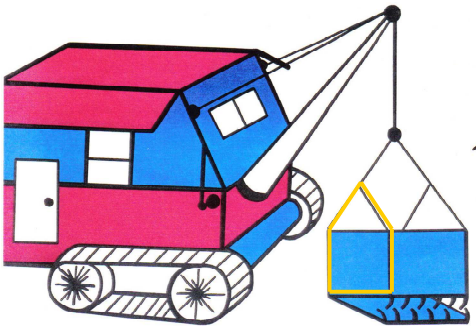
Figure 2.1. Sample Children's Embedded Figures Items



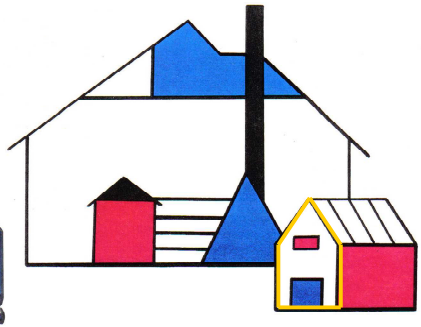
CEFT Tent Test Item 1



CEFT Tent Test Item 11



CEFT House Test Item 6



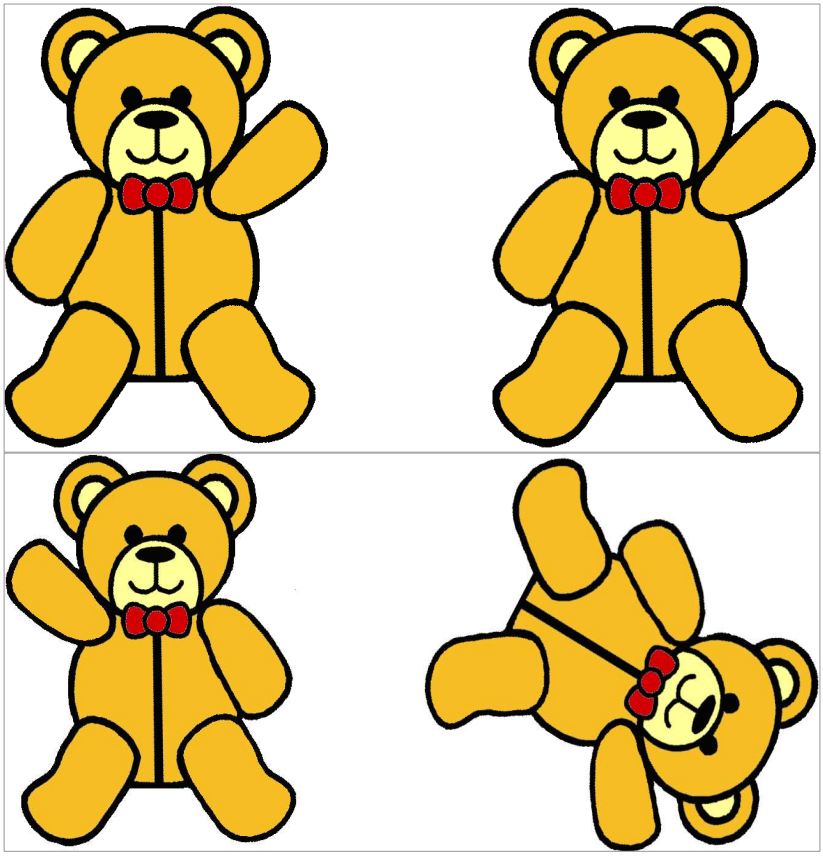
CEFT House Test Item 12

Targeting. In this task, children used an overhand throw to try to hit the centre of a target mounted on a wall (elevated 150 cm and at a distance of 2 metres) with a tennis-sized ball. This was an adapted dart-throwing task (for use with young children) where the ball sticks to the target. Ten trials were performed with one hand followed by ten trials with the other hand. The order of the hands was counterbalanced across participants. Each throw was scored for its horizontal and vertical deviation from the centre of the target, and the radial error was computed from these scores. Overall scores for each hand consisted of the total radial error measure for ten trials, and the mean of the right hand and left hand scores was calculated. A score of 100 was given for trials where the child hit the centre of the target, and a score of zero was given in the case of the child completely missing the target.

Mental Rotation (MR). This computer-presented mental rotation task comprised of two teddy bears displayed simultaneously (Grimshaw et al., 1995b). The bear on the right-hand side of the screen is presented upright or rotated (30, 60, 90, 120, 150, 180

degrees), while the left-hand bear remained upright. The child indicates if the teddy bears are holding up the 'same' or 'different' arm by pressing one of two buttons. Half of the presentations showed the teddy bear raising the same arm, and half of the presentations showed the bear holding up a different arm. Practice items and a criterion test were administered prior to the experimental test. Equal numbers of same and different items were presented in random order and the child met criterion if they responded correctly on any 20 of 24 trials or if they responded correctly on 10 consecutive items. A total of four children did not meet criterion and did not complete the computer task. The experimental procedure included pre-training on same/different judgements (4 trials), criterion test (10-24 trials), pre-training on bear rotation (10 trials) and the experimental test (46 trials). Administration of this task required approximately twenty minutes to complete. Two measures of MR were taken: the mean time the child took to respond (recorded in milliseconds) and the number of correct responses. See Figure 2.2.

Figure 2.2. Sample Mental Rotation Items



2.2.3. *Predictor variables*

Foetal Testosterone (FT) levels. The major predictor in this study is FT level in amniotic fluid, measured by radioimmunoassay. Amniotic fluid was extracted with diethyl ether. The ether was evaporated to dryness at room temperature and the extracted material re-dissolved in an assay buffer. Testosterone was assayed by the DPC 'Count-a-Coat' method (Diagnostic Products Corp, Los Angeles, CA 90045-5597), which uses an antibody to testosterone coated onto propylene tubes and a 125-I labelled testosterone analogue. Units of foetal testosterone are expressed in nanomoles per litre (nmol/L). The detection limit of the assay using the ether-extraction method is approximately 0.05 nmol/L. The coefficient of variation (CV) for between batch imprecision is 19% at a concentration of 0.8 nmol/L and 9.5% at a concentration of 7.3 nmol/L. The CV's for within batch imprecision are 15% at a concentration of 0.3 nmol/L and 5.9% at a concentration of 2.5 nmol/L. This method measures total extractable testosterone.

The following control variables were included in all subsequent analyses.

Gestational age at amniocentesis (in weeks). The amniocentesis procedure generally occurs between weeks 14 and 22. Therefore it is important to determine whether FT is related to gestational age.

Maternal age. Maternal age was included because women undergoing amniocentesis have a higher mean age than the general childbearing population.

Level of education obtained by parents. The mean maternal and paternal education level was computed. Parental education level was measured according to a 5-point scale: 1 = no formal qualifications, 2 = O level/GCSE or equivalent, 3 = A level, HND or vocational qualification, 4 = university degree and 5 = postgraduate qualification.

Presence of older siblings. Older siblings have been found in previous research to have an impact on the social environment and influence child development (Nystul, 1981). This

variable was defined as: older brothers present in the home (or not) and older sisters present in the home (or not).

Child's Age. The children included in the analyses were between 6 and 10 years of age, and child's age was included as a control variable.

2.3. Statistical Analyses

For Chapters 2 to 7, the distributions for all outcome variables are examined. Scores showing distributions deviating from the Gaussian distribution will be transformed as appropriate.

Sex-differences are examined using independent samples t-test. The existence of sex differences on outcome measures indicates a possible role for FT. Outcome variables that demonstrate significant sex differences are further examined using a hierarchical multiple regression analysis. In the first stage, any predictor variable that shows a significant correlation with the outcome at the $p<0.20$ is entered into the analysis (Altman, 1991). In addition, the influence of suppressor variables (predictors that are highly correlated with other predictors in the model at $p<0.01$) is investigated. The main effects of FT level and child sex are tested for inclusion in the second stage using the stepwise method (entry criterion $p<0.05$, removal criterion $p>0.10$). The interaction between child sex and FT level is tested for inclusion in the third stage using the stepwise method. Correlation coefficients are displayed for girls and boys together, as well as separately.

Effect sizes are also computed using 'Cohen's d'. This is calculated by dividing the difference in means for the two groups by the standard deviation. It provides a standardised measure of the magnitude of group differences that can be compared across samples of different size. A d of .2 to .4 is considered a small effect size. A d

between .5 and .7 is considered a medium effect size. A d greater than .8 is considered a large effect size (Cohen, 1988).

2.4. Results

Examination of the univariate distributions revealed that FT level was positively skewed, and was the only predictor variable with a distribution that deviated significantly from the Gaussian distribution. Two female outliers and one male outlier in FT levels (individuals who scored three or more standard deviations from the mean) were observed. These outlying values were replaced using a windsorizing procedure (Barnet & Lewis, 1978), where the extreme values are replaced by the highest observed level within three standard deviations from the mean (1.00 nmol/L for girls and 1.70 nmol/L for boys). The windsorizing procedure was chosen because it is a compromise between the two goals of eliminating the strong influence of extreme values while at the same time utilising all of the information. Windsorized FT levels showed no outliers and acceptable skewness statistics for both boys and girls, and are used in subsequent analyses.

As expected, results show a strong correlation between measured FT levels and Sex. This lends validity to the use of FT levels from amniotic fluid as a good reflection of foetal exposure to androgen levels.

Table 2.1 presents the means and standard deviations for each sex separately, as well as combined for predictor and outcome variables. Table 2.2 shows the correlation coefficients for both the predictor and outcome variables. Tables 2.3 and 2.4 show correlation coefficients for girls and boys separately.

Table 2.1. Descriptive statistics

	All cases				Girls				Boys				
Variable	n	M	SD	Range	n	M	SD	Range	n	M	SD	range	Cohen's d
^FT level (nmol/L)**	101	0.59	0.40	0.05-1.95	45	0.40	0.38	0.05-1.75	56	0.75	0.36	0.10-1.95	0.95
Gestational Age	64	16.08	1.37	13-20	27	16.19	1.35	14-19	37	16.01	1.41	13-20	0.13
Child Age	101	9.01	0.93	7.01-10.66	45	8.90	1.01	7.01-10.42	56	9.10	0.85	7.03-10.66	0.21
Maternal Age	99	35.32	4.63	23.68-45.90	45	35.33	4.61	23.68-45.66	54	35.31	4.68	25.22-45.90	0.00
Parent Education	98	3.41	.097	2-5	44	3.18	0.79	2-5	54	3.60	1.07	2-5	0.45
Full Scale IQ	74	105.61	15.22	74-142	32	101.75	14.02	74-126	42	108.55	15.60	74-142	0.48
Verbal IQ	74	98.01	15.22	66-142	32	95.53	13.11	70-131	42	99.90	16.56	66-142	0.29
Performance IQ	74	113.03	17.18	80-151	32	108.88	17.62	80-151	42	116.19	16.34	81-151	0.43
Block Design	74	17.93	9.73	1-43	32	16.44	9.97	4-42	42	19.07	9.51	1-43	0.27
EFT**	98	12.29	5.38	2-23	42	10.45	5.12	2-23	56	13.66	5.20	3-23	0.62
Targeting (total)	94	641.95	152.52	200-1225	42	616.73	154.41	250.0-912.5	52	662.33	149.35	200-1225	0.30
MR (mean time)	71	2841.47	689.51	1248.63-4448.33	34	2862.93	714.08	1248.63-4165.90	37	2821.75	675.40	1521.11-4448.33	0.06
MR (correct)	71	41.48	10.96	10-60	34	38.88	12.39	10-60	37	43.86	8.99	26-56	0.46

^Indicates raw values

* Sex difference significant at the $p<0.05$ level

** Sex difference significant at the $p<0.01$ level

Table 2.2.Correlation matrix for all cases

	FT Level	Sex	Gest. Age	Child Age	Matr. Age	Parent Ed.	Older Sister	Older Brother	Full IQ	Verb. IQ	Perf. IQ	Block Design	EF T	Targe-ting	MR Mean
Sex	.52**	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Gest. Age	-.04	-.07	--	--	--	--	--	--	--	--	--	--	--	--	--
Child Age	-.05	.11	-.04	--	--	--	--	--	--	--	--	--	--	--	--
Maternal Age	-.11	-.06	-.32**	.05	--	--	--	--	--	--	--	--	--	--	--
Parent Education	.08	.22*	-.07	-.01	.07	--	--	--	--	--	--	--	--	--	--
Older Sister	.04	-.03	-.16	-.01	.14	-.07	--	--	--	--	--	--	--	--	--
Older Brother	-.02	-.13	-.19	-.27	-.06	-.15	.42**	--	--	--	--	--	--	--	--
Full Scale IQ	.10	.22	-.20	.12	-.03	.25*	-.23*	-.31**	--	--	--	--	--	--	--
Verbal IQ	-.01	.14	-.16	.14	-.01	.18	-.20	-.32**	.83**	--	--	--	--	--	--
Perf. IQ	.19	.21	-.18	.06	-.05	.25*	-.20	-.20	.84**	.40**	--	--	--	--	--
Block Design	.19	.14	-.10	.31**	.01	.19	-.26*	-.23*	.68**	.27*	.85**	--	--	--	--
EFT	.57**	.30**	-.16	.16	.06	.15	.07	.02	.31**	.15	.37**	.37**	--	--	--
Targeting (total)	.11	.15	.04	.44**	.01	.07	-.03	-.01	.17	.07	.22	.42**	.16	--	--
MR (mean time)	-.10	-.03	.11	-.19	.15	.02	-.07	.06	.25	.21	.24	.18	.12	-.12	--
MR (correct)	.14	.23	.16	.34**	-.10	.09	.04	-.20	.27*	.33*	.14	.12	.26*	.07	-.02

* $p<0.05$, ** $p<0.01$

Table 2.3. Correlation matrix for Girls

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Ed.	Older Sister	Older Brother	Full IQ	Verb. IQ	Perf. IQ	Block Design	EFT	Targe-ting	MR Mean
Gest. Age	.08	--	--	--	--	--	--	--	--	--	--	--	--	--
Child Age	-.04	.12	--	--	--	--	--	--	--	--	--	--	--	--
Maternal Age	-.09	-.50**	-.20	--	--	--	--	--	--	--	--	--	--	--
Parent Education	-.22	.10	.02	-.08	--	--	--	--	--	--	--	--	--	--
Older Sister	.10	-.03	-.09	-.04	-.08	--	--	--	--	--	--	--	--	--
Older Brother	.11	-.25	-.37*	.05	-.22	.44**	--	--	--	--	--	--	--	--
Full Scale IQ	.20	-.09	.12	-.04	-.03	-.14	-.22	--	--	--	--	--	--	--
Verbal IQ	-.03	.04	.07	-.02	-.10	-.16	-.24	.77**	--	--	--	--	--	--
Perf. IQ	.33	-.21	.15	-.03	.02	-.10	-.15	.86**	.36*	--	--	--	--	--
Block Design	.38*	-.09	.38*	.07	.03	-.26	-.28	.74**	.29	.89**	--	--	--	--
EFT	.70**	-.20	.20	.08	-.08	.18	.08	.29	.04	.43*	.48**	--	--	--
Targeting (total)	.17	.09	.58**	-.11	.09	-.01	-.10	.31	.26	.29	.49**	.14	--	--
MR (mean time)	-.04	-.10	-.23	.36*	-.22	-.05	.29	-.07	-.06	-.04	-.05	.05	-.25	--
MR (correct)	.08	.31	.44**	-.32	-.25	.07	-.23	.39	.43*	.25	.28	.20	.15	-.21

* $p<0.05$, ** $p<0.01$

Table 2.4. Correlation matrix for Boys

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Ed.	Older Sister	Older Brother	Full IQ	Verb. IQ	Perf. IQ	Block Design	EF T	Targe-ting	MR Mean
Gest. Age	-.05	--	--	--	--	--	--	--	--	--	--	--	--	--
Child Age	-.19	-.17	--	--	--	--	--	--	--	--	--	--	--	--
Maternal Age	-.17	-.35*	.06	--	--	--	--	--	--	--	--	--	--	--
Parent Education	.04	-.15	-.07	.16	--	--	--	--	--	--	--	--	--	--
Older Sister	.04	-.22	.09	.20	-.06	--	--	--	--	--	--	--	--	--
Older Brother	.02	-.14	-.11	.00	-.06	.40**	--	--	--	--	--	--	--	--
Full Scale IQ	-.16	-.20	.14	-.07	.35*	-.30	-.38*	--	--	--	--	--	--	--
Verbal IQ	-.15	-.26	.19	-.02	.29	-.23	-.39*	.86**	--	--	--	--	--	--
Perf. IQ	-.08	-.10	.01	-.11	.33*	-.29	-.24	.82**	.41**	--	--	--	--	--
Block Design	-.06	-.03	.26	-.08	.24	-.26	-.19	.62**	.24	.82**	--	--	--	--
EFT	.43**	-.14	.08	.09	.21	.00	.03	.23	.15	.21	.22	--	--	--
Targeting (mean)	-.06	.01	.31*	.12	.01	-.03	.10	.00	-.11	.10	.33*	.08	--	--
MR (mean time)	-.09	.29	-.14	-.10	.20	-.08	-.20	.48**	.37*	.47**	.38*	.23	-.02	
MR (correct)	-.06	.04	.18	.17	.32	-.01	-.12	.03	.19	-.13	-.15	.16	-.05	.24

* $p<0.05$, ** $p<0.01$

2.4.1. *IQ and Block Design*

Table 2.5 shows the mean, standard deviation and t-test results for IQ scores. No significant sex differences were found between boys and girls for Full Scale IQ, Performance IQ, Verbal IQ or Block Design scores (using the Bonferroni correction for multiple comparisons).

Table 2.5. IQ descriptive statistics

	Girls (n=32)		Boys (n=42)		
Variable	M	SD	M	SD	t
Full Scale IQ	100.61	13.86	108.95	15.64	2.37
Verbal IQ	94.90	12.82	100.26	15.52	1.51
Performance IQ	108.13	17.39	116.26	16.70	2.03
Block Design	16.26	10.08	19.14	9.41	1.26

Note: All t-tests were non-significant

No significant correlations were found between any of the predictor variables and Full Scale IQ, Performance IQ, Verbal IQ or Block Design scores. Regression analyses were not conducted for these variables.

2.4.2. *Children's Embedded Figures Test (EFT)*

For EFT score, examination of the univariate distribution revealed that it was not skewed (skewness<1) for all participants together as well as for boys and girls separately. A sex difference was found in EFT scores with boys (M=13.66, SD=5.20) scoring higher than girls (M=10.45, SD=5.12), $t(96)=3.04$, $p<0.01$ (equal variances assumed). See Figure 2.3 for the distribution of EFT scores.

Figure 2.3. Distribution of EFT scores

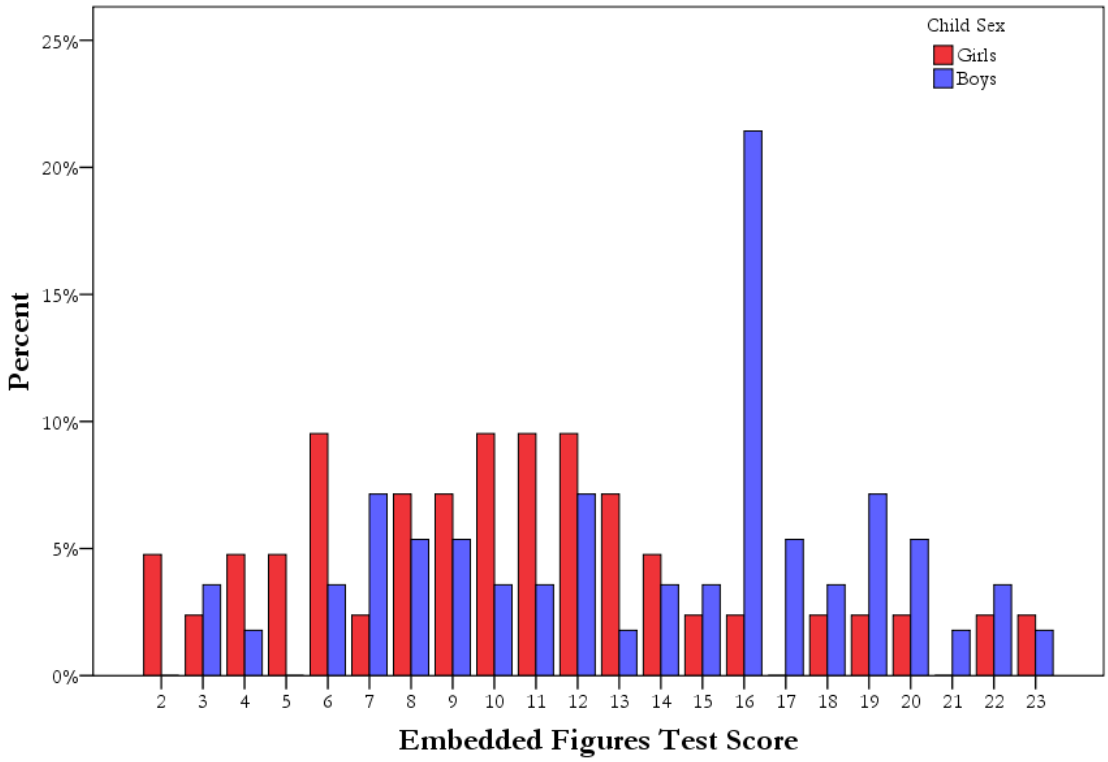
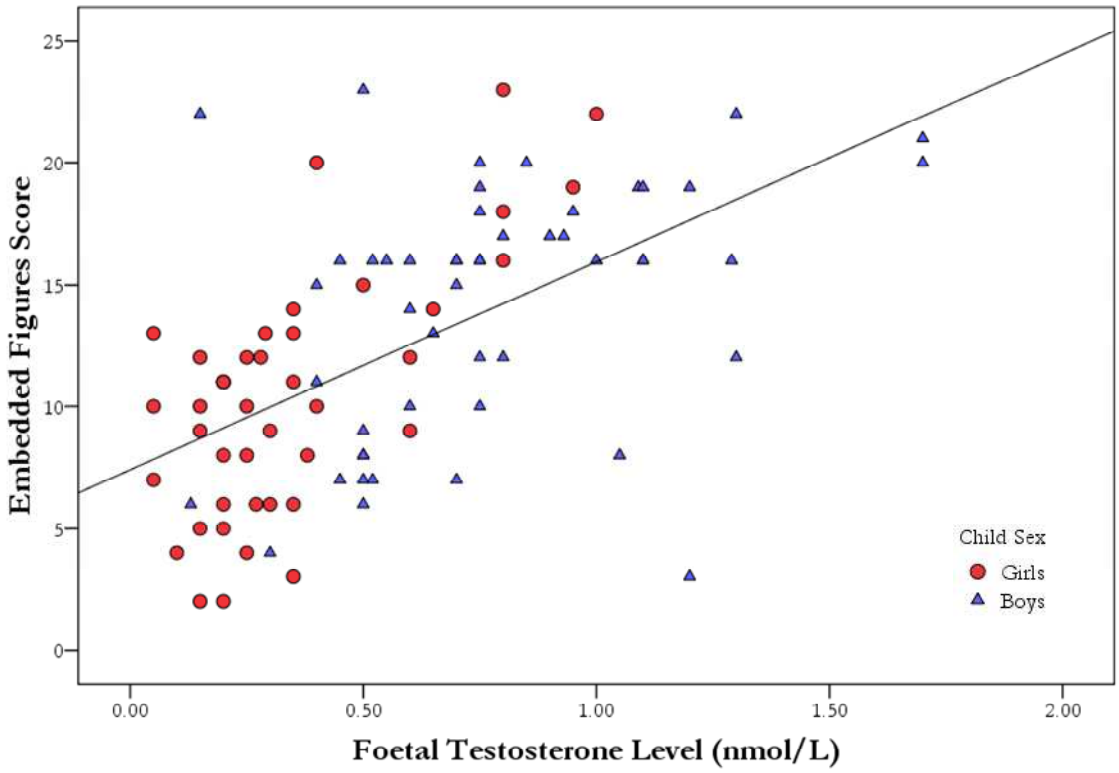


Table 2.6. Final regression model for EFT scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
EFT	Child age	0.05	0.05	1.30	0.48	0.22	p<0.01
	Parent education			0.82	0.47	0.15	p>0.05
	FT level	0.38	0.33	11.69	1.73	0.78	p<0.001
	FT level X Sex	0.42	0.04	2.54	1.04	0.29	p<0.05
		Girls only					
EFT	Child age	0.04	0.04	1.19	0.54	0.24	p<0.05
	FT level	0.54	0.50	15.04	2.31	0.71	p<0.001
		Boys only					
EFT	Parent education	0.04	0.04	0.93	0.61	0.19	p<0.001
	FT level	0.23	0.18	6.53	1.88	0.43	p<0.001

Table 2.7 shows regression results for boys and girls together as well as separately. The predictor variables that correlated with EFT scores at $p<0.20$ was child age ($r=0.16$, $p<0.20$) and parent education ($r=0.15$, $p<0.20$) and were included in the regression analysis using the enter method in the first stage. The inclusion of FT level in the second stage produced a significant F-change (F-change=66.76, $p<0.001$, $\Delta R^2=0.33$). Inclusion of the Sex/FT level interaction also produced a significant F-change (F-change=8.26, $p<0.01$). Child sex was excluded as a predictor from the final regression model. See Figure 2.4 for a visual representation of the relationship between FT level and EFT scores for males and females combined.

Figure 2.4. FT level and EFT scores



Within sex analyses showed that FT levels were significantly related to EFT scores in girls ($r=0.70$, $p<0.05$) and boys ($r=0.43$, $p<0.05$). The regression analysis for girls showed that the inclusion of FT level in the second stage produced a significant F-change (F-change=99.78, $p<0.001$, $\Delta R^2=0.50$). The regression analysis for boys also

showed a significant F-change (F-change=12.09, $p<0.01$, $\Delta R^2=0.18$) when FT level was included in the second stage.

2.4.3. *Targeting*

Examination of the univariate distribution for targeting revealed that it was not skewed (skewness<1) for all cases together as well as in boys and girls separately. No significant sex differences were found between boys (M=616.73, SD=154.41) and girls (M=662.33, SD=149.35) for targeting ($t(92)=1.45$, $p>0.05$, equal variances assumed). Targeting did not show significant correlations with any of the predictor variables (all $p>0.05$), therefore regression analyses were not conducted for targeting score.

2.4.4. *Mental Rotation (MR)*

Examination of the univariate distribution for MR revealed that it was also not skewed (skewness<1) for all cases together as well as in boys and girls separately. No significant sex differences were found between boys (M=2821.75, SD=675.40) and girls (M=2862.93, SD=714.08) for MR mean time ($t(69)=0.25$, $p>0.05$, equal variances assumed). The difference in MR correct score between boys (M=43.86, SD=8.99) and girls (M=38.88, SD=12.39) was also not significant, $t(69)=1.95$, $p>0.05$, equal variances assumed. No significant associations between MR score and any of the predictor variables were observed. Thus, regression analyses were not conducted.

2.5. Discussion

In this study, the main predictor variable of FT was found to be significantly related to Sex and also to performance on the Children's Embedded Figures Test (EFT). For EFT, this relationship was found when girls and boys were examined together as well as independently. The findings from the EFT are in accordance with the correlation between EFT performance and 2D:4D ratio in adults since lower 2D:4D ratios have been found to be associated with a higher ratio of FT levels and foetal oestradiol level

(Lutchmaya et al., 2004). Results are also consistent with the findings that girls with CAH exhibit masculinised performance for figure disembedding (Resnick et al., 1986). The high values of the correlations and the significant relationships observed between FT levels and EFT score provide evidence for a link between foetal androgen exposure and spatial ability. Results also show a clear link between EFT scores and Sex, with males scoring higher. This is consistent with previous research which also suggests superior male performance (Kimura, 1999; Nebot, 1988). Findings in adults also suggest that adults with ASC are superior compared to controls for this task (Jolliffe & Baron-Cohen, 1997).

Other findings suggest that FT level is not related to individual variation in Full Scale IQ, Verbal IQ, Performance IQ or Block Design scores. No sex differences were found for these variables. If the effect of normal variation in FT level on IQ is small, then only studies with large sample sizes will reveal it. It is also possible that FT levels contribute to IQ but does so at a different time period than that examined in this study. However, the lack of significant sex differences in IQ scores in the current study suggest that even in a larger sample, no relationship between FT levels and IQ (and Block Design performance) would be observed. It would be beneficial for future studies to examine the relationship between FT levels and IQ scores (in particular block design performance) in a larger sample of children. It would also be interesting to examine if these relationships remain consistent throughout adolescence and adulthood, since sex differences in Block Design performance have been found in adults (Lynn, 1998; Rönnlund & Nilsson, 2006).

Studies have shown that children with ASC show superior performance compared to controls on the EFT and Block Design subtest. In Shah and Frith's (1983) study of EFT performance, children with ASC performed significantly better than either typically developing children or children with mental retardation. These results suggest that children with ASC score at a similar level to children of the same age. In contrast, although children with ASC show better performance on tasks such as the Block Design

and Object Assembly subtests than on verbal subtests, their performance is usually below their age level (Shah & Frith, 1983). Shah and Frith (1983) propose that children with ASC may show good 'orientation' ability (defined as the ability to arrange elements within a pattern and demonstrated by good embedded figures performance) but poor 'visualisation' (involving the ability to manipulate, rotate, twist or invert an object as required for tasks such as Block Design). It is also possible that the effects of FT are only observed in the analytic/orientation component and not for the visualisation/rotational/spatial elements. Further examination of this possibility is clearly warranted.

For targeting, no significant sex differences and no significant associations with FT were found. However, targeting scores did show an association with child age. Falter et al. (2008) found that individuals with ASC did not show superior targeting performance compared to typically developing children. It is possible that problems with motor coordination in the ASC group (which are unrelated to FT exposure) affected their abilities on this task (Knickmeyer et al., 2008). Regardless of these possible motor difficulties, results suggest that this is not an area where children with ASC show an advantage compared to typically developing controls (Falter et al., 2008). This shortage of evidence linking targeting and ASC may possibly explain the lack of an observed relationship between FT levels and targeting. Conversely, other studies have found that females with CAH perform better than control females in targeting (Hines et al., 2003b). It is possible that the task used in the current study was a poor measure of targeting in this sample, suggested by the significant positive correlation between targeting and child age ($r=0.44$, $p<0.01$). It may be that this task was too difficult for the younger children. Future studies should examine whether the current results remain consistent as the children progress into adolescence and adulthood.

Sex differences have been found in previous studies of mental rotation ability in adults (Linn & Petersen, 1986; Voyer et al., 1995). However, no significant sex differences or associations were found for mental rotation in this study. These findings were consistent

with previous studies of mental rotation showing no relationships with 2D:4D ratio (Falter et al., 2006). The current findings are also consistent with other evidence which suggests that girls with elevated exposure to FT as a result of CAH are not faster or more accurate at mental rotation (Hines et al., 2003b; Malouf et al., 2006). In the context of the results from mental rotation and targeting, one possibility is that FT plays a role in attention to detail/analysis, but not 'spatial ability'. Hines (2003b) argues that differences in mental rotation may arise from sex differences in neonatal testosterone rather than prenatal levels. The current findings are consistent with this hypothesis. While it is possible that prenatal and neonatal levels are correlated, this has not been tested experimentally.

Although several studies have reported sex differences in spatial ability, some of these differences might be attributed to the activational effects of hormones (i.e. due to circulating hormone levels) in later life. One study examined the relationships between current serum testosterone levels (measured at three six-month intervals) and spatial ability (measured using a mental rotation and Block Design task) in 108 adolescents between 9 and 14 years of age (Davison & Susman, 2001). For boys, higher levels of testosterone (for all three times of measurement) were significantly associated with higher mental rotation and Block Design scores. For girls, higher mental rotation scores were significantly related to testosterone levels only at the third time period, which was when the girls' testosterone levels were at their highest. The researchers suggest that findings might implicate activational effects of testosterone on the mental rotation and Block Design components of spatial ability (Davison & Susman, 2001). No information was available about the effects of prenatal FT for this sample.

In this study, a range of methods were used to evaluate the possibility of a link between prenatal FT levels and spatial ability, measured in children between 6 and 10 years of age. In summary, the results suggest that FT is significantly related to EFT performance, but not to mental rotation, targeting, general intelligence or Block Design performance. A strong link between EFT performance and Sex was also observed. These findings are

consistent with previous studies which have shown a male advantage for this task. Other results reported in this study did not show sex differences, so were not expected to correlate with FT levels. The design of suitable evaluation methods for spatial ability in children is difficult. It is possible that the absence of a link between FT and scores on other measures may be partly due to test design and lack of sufficient power. The positive correlation between targeting score and child age points towards this.

The EMB theory hypothesises that ASC may be an extreme manifestation of certain sexually dimorphic traits, particularly in empathising and systemising. The current findings are consistent with this hypothesis, given the superior performance that children with ASC show on the EFT, and assuming systemising requires good attention to detail as measured on the EFT. Arguably, both mental rotation and Block Design require rotation of mental imagery and it may be that this aspect of spatial skills is unrelated to systemising and may be under the control of a different mechanism to FT. Targeting may also be a poor measure of systemising since it requires aspects of motor performance which may be impaired in individuals with ASC (Knickmeyer et al., 2008). Further research with larger sample sizes is needed to clearly delineate the effects of prenatal and postnatal hormone exposure on the later development of spatial ability in individuals with and without a clinical diagnosis before firm conclusions can be drawn.

Chapter 3: Foetal testosterone and gender-typical behaviour

Sex differences are reported in several areas of behaviour in children. This chapter investigates whether FT is related to childhood gender-typical behaviour in n=207 children. Sex differences were observed for both measures of gender-typical behaviour and their subcomponents. Results indicate a positive association between FT and male-typical scores on a standardised questionnaire measure of gender-typical play when the sexes were combined and in girls alone but not boys alone. These associations were consistent for both subcomponents of this scale. A positive association in girls was also observed between FT and masculinity scores but not femininity scores. These results suggest that FT is involved in shaping gender-typical behaviour.

3.1. Introduction

In animals, manipulating androgens prenatally or neonatally has been shown to permanently alter brain regions and behaviours that show sex differences (De Vries & Simerly, 2002; Goy & McEwen, 1980; Hines, 2004). One example comes from sex-typical play in juvenile mammals. For both rodents and non-human primates, treating developing females with testosterone or other androgens tends to increase male-typical play, whereas reducing androgens in developing males decreases it (Goy & McEwen, 1980; Hines, 2004). The aim of this study is to examine if any relationships exist between gender-typical behaviour and its relationship to FT levels in humans.

3.1.1. *Gender-typical play*

Social interactions are known to play an important role in the development of gender-typical play and toy choices. For example, Fagot (1978) found that boys are encouraged by parents to play with masculine-typical toys and discouraged from playing with feminine-typical toys. Girls, on the hand, are also encouraged to play with feminine-typical toys but not necessarily discouraged from playing with masculine-typical toys (Fagot, 1978). Despite the possible social influences (such as parental encouragement and shaping) that may affect toy preferences, sex differences have been seen in children as young as 12 months (Servin, Bohlin & Berlin, 1999; Snow, Jacklin & Maccoby, 1983). Sex differences have also been found in playmate and activity preferences, and grow larger as children progress into middle childhood (Golombok & Hines, 2002).

In humans, separating the effects of biological and social influences can be complex, making the interpretation of empirical findings difficult. Findings in a study of vervet monkeys has shown that sex differences do exist in this species, similar to those observed in human children (Alexander & Hines, 2002). The proportion of contact time in male vervet monkeys was greater with toys that are typically preferred by boys (a car and a ball), compared to female vervet monkeys who showed greater contact time with

toys that are typically preferred by girls (a doll and a pot) (Alexander & Hines, 2002). Contact time with toys preferred equally by boys and girls (a picture book and a stuffed dog) did not differ between male and female vervet monkeys.

The strongest evidence that androgens influence human sexual differentiation comes from studies of play behaviour in girls exposed to abnormally high levels of androgens because of Congenital Adrenal Hyperplasia (CAH), a genetic disorder that causes excess adrenal androgen production beginning prenatally (New, 1998). Several research groups have reported that girls with CAH show increased male-typical toy, playmate and activity preferences (Ehrhardt & Meyer-Bahlburg, 1981; Hines, 2003; Hines, 2004; Pasterski et al., 2005). Because girls with CAH are treated postnatally to normalise hormones, this behavioural masculinisation is thought to result from prenatal androgen exposure. However, some researchers have proposed that it is the CAH-related disease characteristics, rather than prenatal androgen exposure, that could be responsible for these patterns of behaviour (Fausto-Sterling, 1992; Quadagno et al., 1977).

Studies relating prenatal testosterone to play behaviour in typically developing children have produced mixed results. One study, based on a large population sample, examined gender role behaviour using the Pre-School Activities Inventory (PSAI) in relation to testosterone levels measured in maternal blood samples from pregnant women (Hines et al., 2002a). The researchers reported a positive relationship between maternal testosterone during pregnancy and male-typical play in girls, but not boys (Hines et al., 2002a). It is possible that this relationship could reflect mothers with high testosterone encouraging more male-typical play in their daughters, rather than an effect of testosterone on the developing brain (Cohen-Bendahan et al., 2005a; Hines et al., 2002a). However, the results from this study suggest that normal prenatal testosterone variation may contribute to the development of individual differences in the gender role behaviour of girls (Hines et al., 2002a).

Prenatal hormonal effects on gender-typical development in children have also been investigated in opposite-sex twins. It is hypothesised that opposite-sex dizygotic twins will show less stereotyped play due to the effects of transfer of hormones their twins produce in utero. Contrary to the expected relationship, evidence suggests that girls with a boy co-twin do not spend more time playing with boys' toys compared to girls with a girl co-twin, and vice versa (Henderson & Berenbaum, 1997; Rodgers, Fagot & Winebarger, 1998b). The difference between findings in these opposite-sex twin girls compared to girls with CAH may be evidence for masculinisation only when hormone levels are abnormally high. However, a drawback of using opposite-sex twins to examine the effects of hormones is that the transfer of hormones is assumed, whilst direct, quantitative measures of hormone levels are not available.

Other studies have investigated the relationship between testosterone measured in amniotic fluid and subsequent gender-typical play behaviour (Knickmeyer et al., 2005b; van de Beek et al., 2008). The first study was conducted using the Cambridge FT Project sample and findings in a sample of 53 children showed no relationship between FT levels and scores on a modified version of the Child Game Participation Questionnaire. Another study examining the effects of gender-related play behaviour and prenatal sex hormones (measured in amniotic fluid and in maternal serum collected immediately following amniocentesis) also showed no relation to toy preference in 126 children.

Examination of sex-typical play in children with ASC has shown that they participate in less pretend play compared to typically developing children (Baron-Cohen, 1987; Jarrold, Boucher & Smith, 1993). A potential concern with any measure of sex-typical play applied to children with ASC is that many such games require pretence (or pretend play) (Knickmeyer, Wheelwright & Baron-Cohen, 2007). This creates the possibility that a real change in preference for gender-typical play might be concealed by reduced participation in games that require pretence. Examination of children with ASC showed that girls with this condition did not show female-typical play preferences for games that did not require pretence, providing partial support for the hypothesis that prenatal

masculinisation of the brain increases the risk of developing ASC (Knickmeyer et al., 2007).

3.1.2. *Gender-role identification*

In addition to sex-typical play behaviour, evidence indicates that gonadal steroids are critical in the development of a typical male or female personality (Collaer & Hines, 1995; Ehrhardt & Meyer-Bahlburg, 1981). The determining role of prenatal steroids in sex-role identity appears to be supported by studies of females with CAH who demonstrate a masculine bias on various personality inventories (e.g. Detachment and Indirect Aggression Scales, Aggression and Stress Reaction Scales, Reinish's Aggression Inventory) (Collaer & Hines, 1995). The previously mentioned findings from women with CAH are consistent with the hypothesised association between prenatal steroids and adult sex-role identity, but they provide less evidence as to the role of hormones in determining sex-roles shown by typically developing individuals.

For the Bem Sex Role Inventory (BSRI), a measure of sex-role identity, a significant relationship was observed between lower 2D:4D ratio and higher, more masculinised scores in typical women (Csatho et al., 2003). However, a second study using the short version of the Personal Attributes Questionnaire (Spence, Helmreich & J., 1974), showed no significant associations between 2D:4D ratio and the masculinity or femininity subscales of this measure when controlling for sex (Hampson et al., 2008).

Among women with ASC, one study has found increased rates of self-reported bisexuality or asexuality (Ingudomnukul et al., 2007). In addition, a tendency towards 'tomboyism' in relation to childhood interests and activities was found (Ingudomnukul et al., 2007). These results suggest that women with ASC may exhibit more masculinised or gender-atypical personality profiles.

3.1.3. *Aims*

The current study of 207 typically developing children aims to examine if any relationships exist between FT levels and gender-typical play behaviour utilising the Pre-School Activities Inventory (PSAI). The PSAI has been standardised on more than 2,000 children in the United Kingdom, and has proved to be a sensitive measure of gender role behaviour. It has also been validated by comparing parental ratings to teacher ratings (Golombok & Rust, 1993a). The relationship between FT levels and the psychological dimensions of Masculinity and Femininity measured using a parental-report version of the Bem Sex Role Inventory (BSRI) (Bem, 1974) is also examined. The investigation of play behaviour and gender role identification could help to clarify the role of testosterone in human sexual differentiation, as well as behaviours associated with ASC.

3.2. Methods

3.2.1. *Participants*

The PSAI and BSRI were sent to all mothers meeting inclusion criteria, resulting in 456 mothers contacted; 222 mothers completed the PSAI and 242 mothers completed the BSRI, resulting in a total of 207 (107 boys, 100 girls) children with complete data for both questionnaires.

3.2.2. *Outcome variables*

The Pre-School Activities Inventory (PSAI) (Golombok & Rust, 1993a, 1993b). The PSAI is a psychometric scale developed to assess sex-typical behaviour in children and, in particular, to assess differences within each sex rather than only differences between boys and girls (see Appendix 1). The PSAI has established validity and a test-retest reliability coefficient of 0.64 (Golombok & Rust, 1993a, 1993b). Item content categories include activity preferences, toy and playmate preferences and temperament items. Each

item has a scoring of 1 to 5 (never, hardly ever, sometimes, often and very often). It is scored by first adding the 'male items', subtracting the 'female items' and then multiplying the result by 1.1 (to make the SD for boys and girls separately close to 10) and adding 48.25 (to render the mean close to 50), and was calculated using the following formula:

Score = 48.25 + 1.1 x (sum of 'male items' – sum of 'female items).

Higher scores reflect more masculine behaviour, and a lower score, more feminine behaviour. The sums of the male and female items were examined and treated as subscales of the PSAI (see Appendix 1).

The Bem Sex Role Inventory (BSRI) (Bem, 1974). This is a 60-item (20 feminine, 20 masculine and 20 non-gender related items) questionnaire developed to measure feminine (F) and masculine (M) personality traits, and was adapted for parent-report (see Appendix 2). The dimensions of Masculinity and Femininity are considered to be independent of each other (Bem, 1974). Items were selected as masculine or feminine on the basis of cultural definitions of sex-typed social desirability (Bem, 1974). Unlike the masculine and feminine items, 10 of the gender-neutral items were identified as desirable and the other 10 as undesirable for both sexes. The measure utilises a seven-point Likert scale, ranging from 1 ("Never or almost never true") to 7 ("Always or almost always true"). Participants indicate how well each of the personality characteristics describes their children. Although several scores and classifications can be obtained from the BSRI, only the Masculinity (M) and Femininity (F) scores were utilised in this study. The neutral adjectives functioned only as filler items and, therefore, were excluded from data analyses.

3.2.3. *Predictor variables*

Chapter 2 provides a detailed description of the predictor variables utilised in this study. FT level was the predictor of greatest interest in this study. The control variables that were included in the subsequent analyses were gestational age at amniocentesis, maternal age, level of education obtained by the parents, presence of older siblings and child's age.

3.3. Results

Examination of the univariate distributions revealed that FT level was positively skewed, and was the only predictor variable with a distribution that deviated significantly from the Gaussian distribution. Two female outliers in FT levels (individuals who scored three or more standard deviations from the mean) were observed. These outlying values were replaced using a windsorizing procedure, where the extreme values are replaced by the highest observed level within three standard deviations from the mean (0.95 nmol/L for girls). No outliers were found when boys' FT levels were examined. Windsorized FT levels showed no outliers and acceptable skewness statistics for both boys and girls, and are used in subsequent analyses.

Table 3.1 presents the means and standard deviations for each sex separately, as well as combined for predictor variables, BSRI and PSAI scores.

Table 3.2 shows the correlation coefficients for predictor and outcome variables for all cases. Tables 3.3 and 3.4 show correlation coefficients for girls and boys separately.

Table 3.1. Descriptive statistics

	Combined Group				Girls				Boys				
Variable	n	M	SD	Range	n	M	SD	Range	n	M	SD	range	Cohen's d
^FT level (nmol/L)**	207	0.60	0.48	0.05-2.05	100	0.31	0.28	0.05-1.75	107	0.88	0.47	0.13-2.05	1.47
Gestational Age	144	16.51	1.42	13-22	69	16.63	1.39	14-22	75	16.40	1.44	13-20	0.16
Child Age	190	9.43	7.20	6.32-10.67	93	8.81	0.99	7.01-10.67	97	9.01	0.93	6.32-10.66	0.21
Maternal Age	180	41.08	4.49	29.42-53.23	87	41.35	4.48	29.42-53.15	93	40.83	4.50	31.67-53.23	0.12
Parental Education	179	3.09	0.98	1-5	88	2.92	0.78	1.50-5.00	91	3.25	1.13	1-5	0.34
PSAI Score**	207	52.52	20.91	13-86	100	35.04	13.09	13-78	107	68.86	11.11	24-86	2.79
PSAI Female Sum**	207	31.63	12.24	13-56	100	41.40	8.92	15-56	107	22.50	6.53	13-47	1.81
PSAI Male Sum**	207	35.51	8.83	17-55	100	29.39	6.23	17-48	107	41.23	6.86	17-55	1.81
BSRI F Score**	207	4.67	0.67	2.35-6.20	100	4.86	0.66	2.85-6.20	107	4.50	0.67	2.35-5.80	0.54
BSRI M Score**	207	4.51	0.81	2.00-6.30	100	4.37	0.69	2.35-6.05	107	4.65	0.89	2-6.30	0.35

^Indicates raw values

* Sex difference significant at the $p<0.05$ level

** Sex difference significant at the $p<0.01$ level

Table 3.2. Correlation matrix for all cases

	FT Level	Sex	Gest. Age	Child Age	Matr. Age	Parent Ed.	Older Sister	Older Brother	PSAI Total	PSAI Fem Sum	PSAI Male Sum	BSRI F Score
Sex	.62**	--	--	--	--	--	--	--	--	--	--	--
Gestational Age	-.04	-.08	--	--	--	--	--	--	--	--	--	--
Child Age	-.01	-.06	.01	--	--	--	--	--	--	--	--	--
Maternal Age	-.05	-.05	-.32**	-.05	--	--	--	--	--	--	--	--
Parent Education	.12	.17*	-.10	.13	.12	--	--	--	--	--	--	--
Older Sister	-.02	-.03	-.12	-.03	.01	-.06	--	--	--	--	--	--
Older Brother	-.01	-.04	-.10	-.03	.07	.01	.30**	--	--	--	--	--
PSAI Total Score	.63**	.81**	-.10	.09	-.04	.16*	.01	-.03	--	--	--	--
PSAI Female Sum	-.58**	-.77**	.03	-.12	.02	-.14	.09	.08	-.93**	--	--	--
PSAI Male Sum	.54**	.67**	-.16	.02	-.05	.14	.03	.05	.56**	-.60**	--	--
BSRI F Score	-.05	-.26**	.20*	-.09	-.01	-.31**	.20**	.07	-.30**	.28**	-.25**	--
BSRI M Score	.27**	.18**	-.07	.07	.06	.04	-.04	-.04	.26**	-.24**	.23**	.06

* $p<0.05$, ** $p<0.01$

Table 3.3. Correlation matrix for Girls

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Ed.	Older Sister	Older Brother	PSAI Total	PSAI Fem Sum	PSAI Male Sum	BSRI F Score
Gestational Age	.16	--	--	--	--	--	--	--	--	--	--
Child Age	-.02	.07	--	--	--	--	--	-	--	--	--
Maternal Age	-.07	-.28*	.06	--	--	--	--	--	--	--	--
Parent Education	-.03	.13	-.11	.06	--	--	-	--	--	--	--
Older Sister	.00	-.05	-.02	-.11	-.11	--	-	--	--	--	--
Older Brother	-.08	.03	.04	-.02	-.14	.36**	--	--	--	--	--
PSAI Total Score	.45**	.06	-.04	.01	.13	.02	-.08	--	--	--	--
PSAI Female Sum	-.44**	-.16	-.06	.06	-.05	-.02	.11	-.86**	--	--	--
PSAI Male Sum	.23*	-.10	-.14	.10	.18	.01	.01	.68**	-.21*	--	--
BSRI F Score	-.02	.20	.03	-.11	-.18	.15	.13	-.19	.12	-.20*	--
BSRI M Score	.24*	-.16	-.07	.11	.08	-.07	-.09	.28**	-.19	.26**	.08

* $p<0.05$, ** $p<0.01$

Table 3.4. Correlation matrix for Boys

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Ed.	Older Sister	Older Brother	PSAI Total	PSAI Fem Sum	PSAI Male Sum	BSRI F Score
Gestational Age	-.03	--	--	--	--	--	--	--	--	--	--
Child Age	-.14	-.05	--	--	--	--	--	--	--	--	--
Maternal Age	.01	-.27*	.17	--	--	--	--	--	--	--	--
Parent Education	.02	-.21	.08	.21*	--	--	--	--	--	--	--
Older Sister	.00	-.18	.02	.11	-.02	--	--	--	--	--	--
Older Brother	.07	-.20	.00	.06	.13	.23*	--	--	--	--	--
PSAI Total Score**	.23*	-.15	.04	-.01	-.05	.10	.13	--	--	--	--
PSAI Female Sum	-.11	.04	-.07	-.11	.02	-.02	.01	-.74**	--	--	--
PSAI Male Sum	.23*	-.17	-.01	-.13	-.05	.12	.20	.77**	-.14	--	--
BSRI F Score	.23*	.17	-.15	.06	-.34**	.24*	-.01	-.10	.13	-.02	--
BSRI M Score	.20*	.02	.15	.05	-.02	-.01	.02	.15	-.15	.08	.14

* $p<0.05$, ** $p<0.01$

3.3.1. *PSAI Scores*

3.3.1.1. PSAI Internal Consistency and reliability

Cronbach's α coefficients were calculated for boys and girls together (α=0.63), however it is important to note that the distribution is bimodal and designed to assess gender-role behaviour (Golombok & Rust, 1993b). Internal consistency for the measure as a whole was satisfactory for girls (α=0.78) and for boys (α=0.76). The internal consistency of the PSAI Female Sum was high for the sexes combined (α=0.93), for girls (α=0.85) and for boys (α=0.84). The internal consistency for PSAI Male Sum was acceptable when the sexes were combined (α=0.86), for girls (α=0.75) and for boys (α=0.73). Split-half reliability was calculated for the sample. For the sexes combined, this was 0.78 (n=207); for girls 0.80 (n=100) and for boys 0.70 (n=107).

Boys (M=68.866, SD=11.11) and girls (M=35.23, SD=13.02) significantly differed in their PSAI scores, $t(205)=20.08$, $p<0.001$ (equal variances assumed).

Figure 3.1. Distribution of PSAI scores

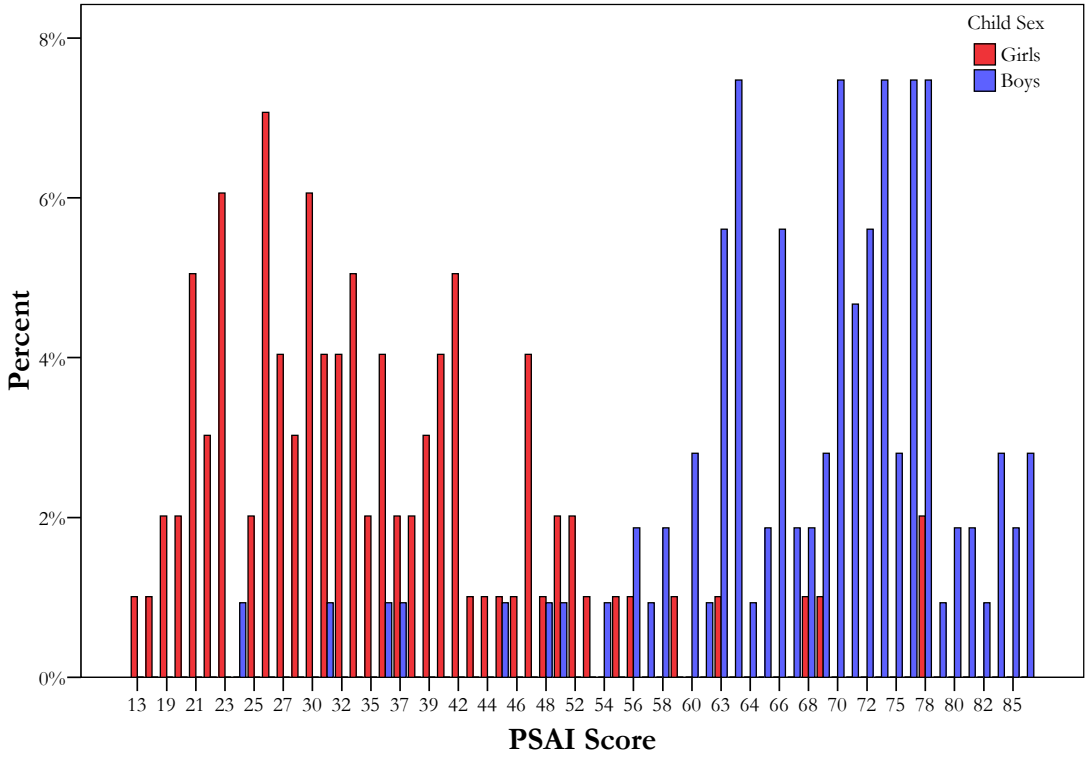
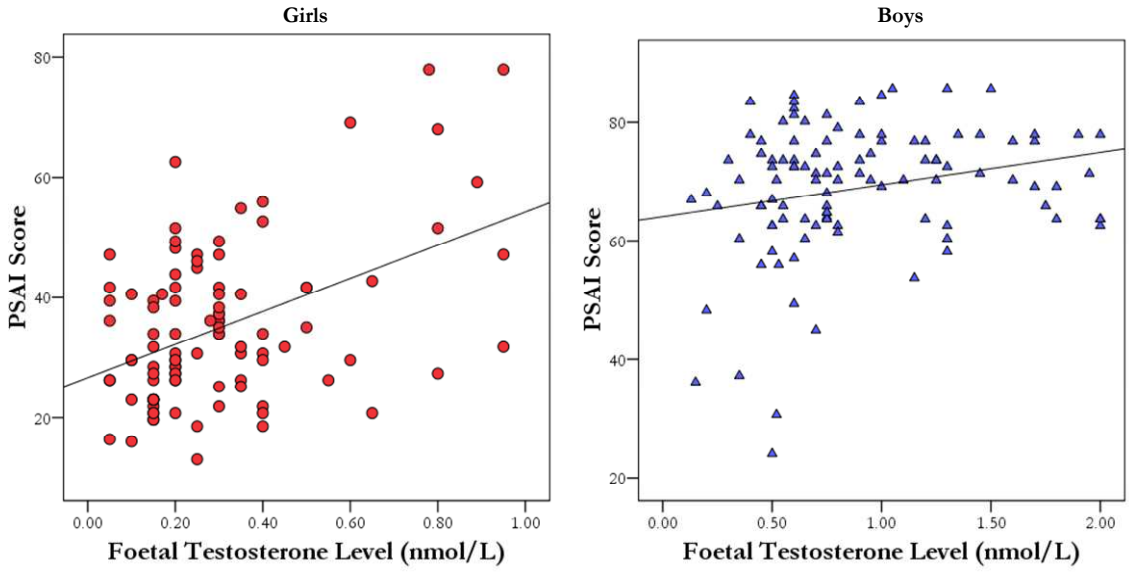


Table 3.5. Final regression model for PSAI Score

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
PSAI Score	Parent education	0.02	0.02	0.35	0.87	0.02	p>0.05
	Sex	0.68	0.66	17.85	1.65	0.86	p<0.001
	FT level	0.71	0.03	16.62	3.39	0.34	p<0.001
	FT level X Sex	0.72	0.01	9.48	3.39	0.29	p<0.01
		Girls only					
PSAI Score	FT level	0.20	0.20	27.57	5.50	0.45	p<0.001
		Boys only					
PSAI Score	Gestational age	.03	0.03	0.92	0.84	0.13	p>0.05
	Older sister			0.34	3.68	0.01	p>0.05
	Older brother			3.26	4.13	0.10	p>0.05

For PSAI scores parent education level (r=0.16, p<0.20) was the only variable that met criteria for inclusion in the first stage of the hierarchical regression analysis. The final model included Sex, FT level and the Sex/FT level interaction in the final regression model (see Table 3.5 for the final regression model). Within sex analyses showed a significant positive relationship between FT level for girls (r=0.45, p<0.001) and boys (r=0.23, p<0.05). For girls, FT level was the only variable that met entry criteria into the regression analysis and was retained in the final model, (F-change =25.11, p<0.001, ΔR^2=0.20). For boys, gestational age (r=-0.15, p<0.20), the presence of older brothers (r=0.24, p<0.20) and sisters (suppressor) were entered in the first stage. FT level was not retained in the final regression model. Figure 3.2 shows the relationship between FT levels and PSAI scores for boys and girls separately.

Figure 3.2. FT level and PSAI scores



3.3.1.2. PSAI Female Sum

A significant sex difference in the Female PSAI scale was found with girls (M=41.40, SD=8.92) scoring higher than boys (M=22.50, SD=6.53), t(180.63)=17.29, p<0.001, equal variances not assumed. See Figure 3.3 for the distribution of scores.

Figure 3.3. Distribution of PSAI Female sum

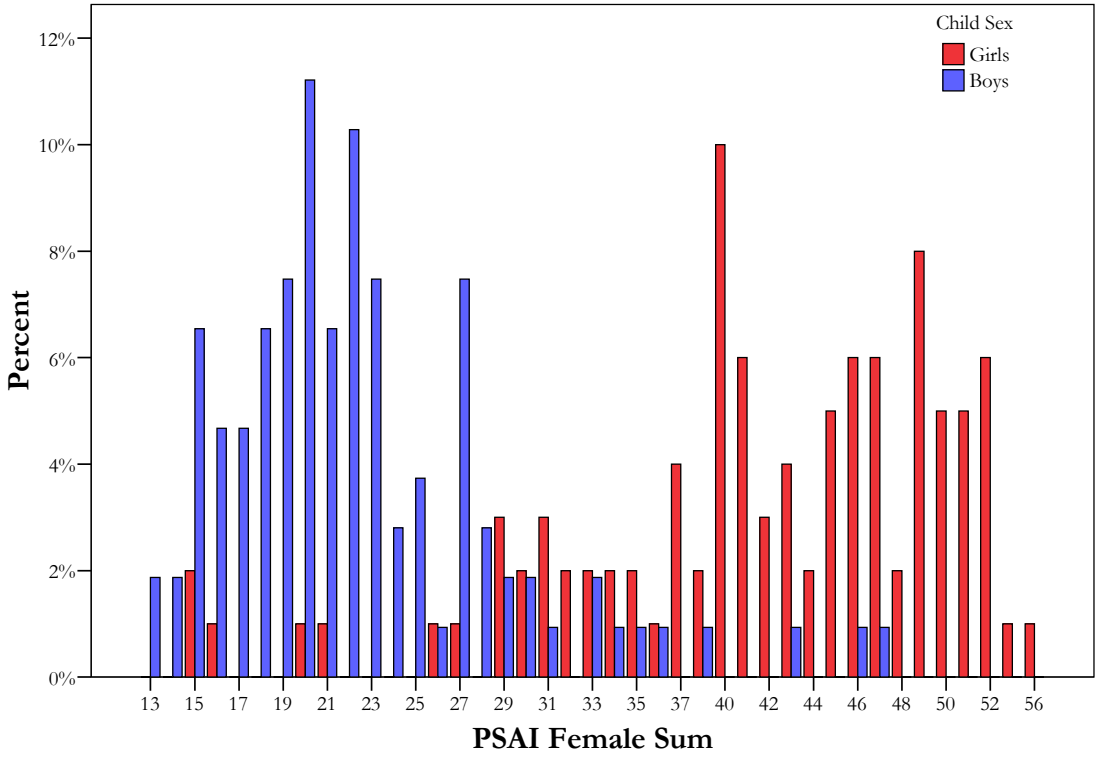
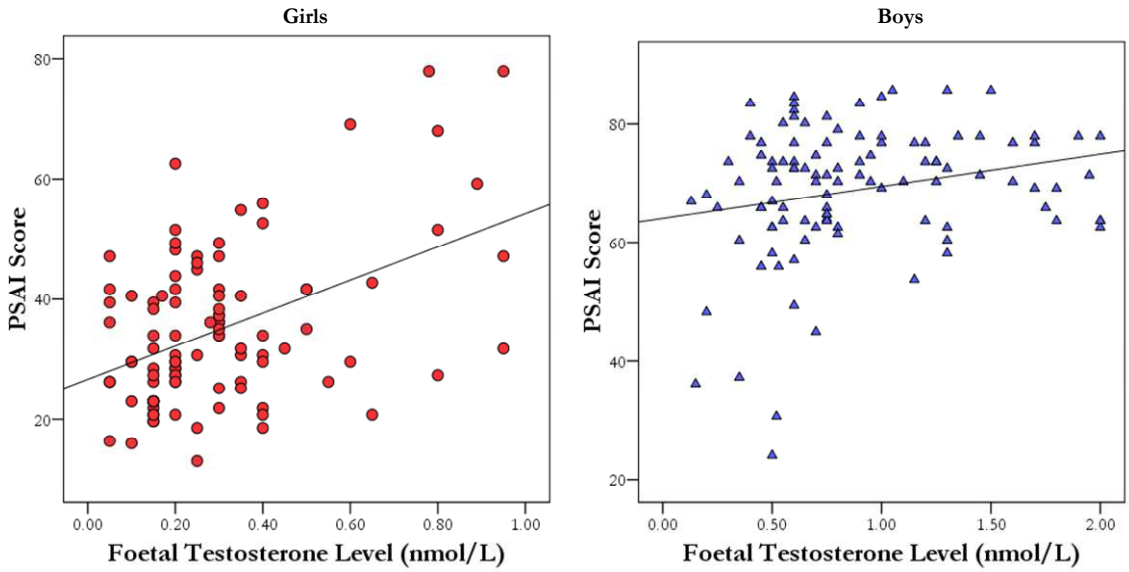


Table 3.6. Final regression model for PSAI Female sum

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
Female Sum	Child age	0.03	0.03	0.46	0.59	0.04	p>0.05
	Parent education			0.02	0.57	0.00	p>0.05
	Sex	0.62	0.59	11.16	1.07	0.92	p<0.001
	FT level	0.63	0.01	8.16	2.18	0.29	p<0.001
	FT level X Sex	0.65	0.02	7.05	2.18	0.37	p<0.01
		Girls only					
Female Sum	Gestational age	0.03	0.03	0.66	0.66	0.12	p>0.05
	FT level	0.10	0.07	11.42	4.94	0.27	p<0.05
		Boys only					
Female Sum	No significant predictors						

For PSAI Female sum, child age (r=-0.12, p<0.20) and parent education level (r=-0.14, p<0.20) were entered into the first stage of the hierarchical multiple regression analysis. The final hierarchical model retained Sex, FT level and the Sex/FT level interaction (shown in Table 3.6). When examining girls alone, gestational age (r=-0.33, p<0.001) was included in the first stage. FT level was retained in the second stage (F-change=3.63, p<0.05, ΔR^2=0.07). In boys, PSAI female sum did not correlate with any of the predictor variables (all p>0.05), therefore a regression analysis was not conducted. Figure 3.4 shows the relationship between FT levels and PSAI Female Sum for boys and girls separately.

Figure 3.4. FT level and PSAI Female sum



3.3.1.3. PSAI Male Sum

The t-test showed a significant sex difference in the Male PSAI scale with boys (M=41.23, SD=6.86) scoring higher than girls (M=29.39, SD=6.53), t(205)=12.98, p<0.001.

Figure 3.5. Distribution of PSAI Male Sum

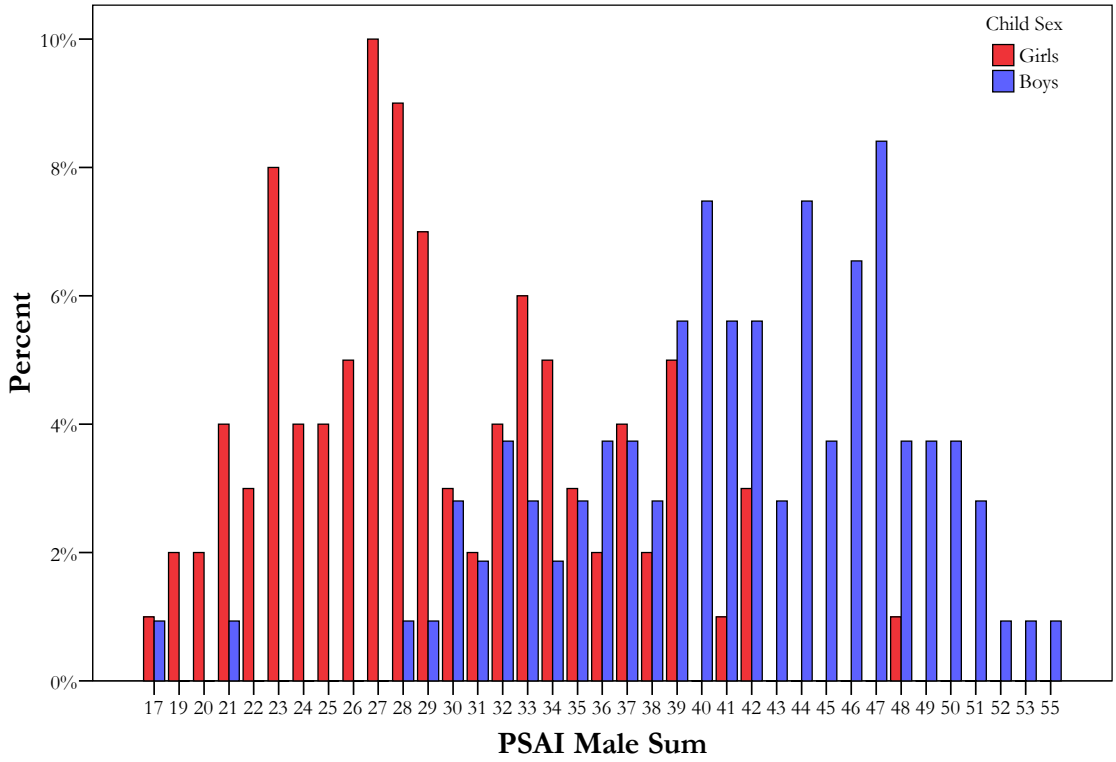
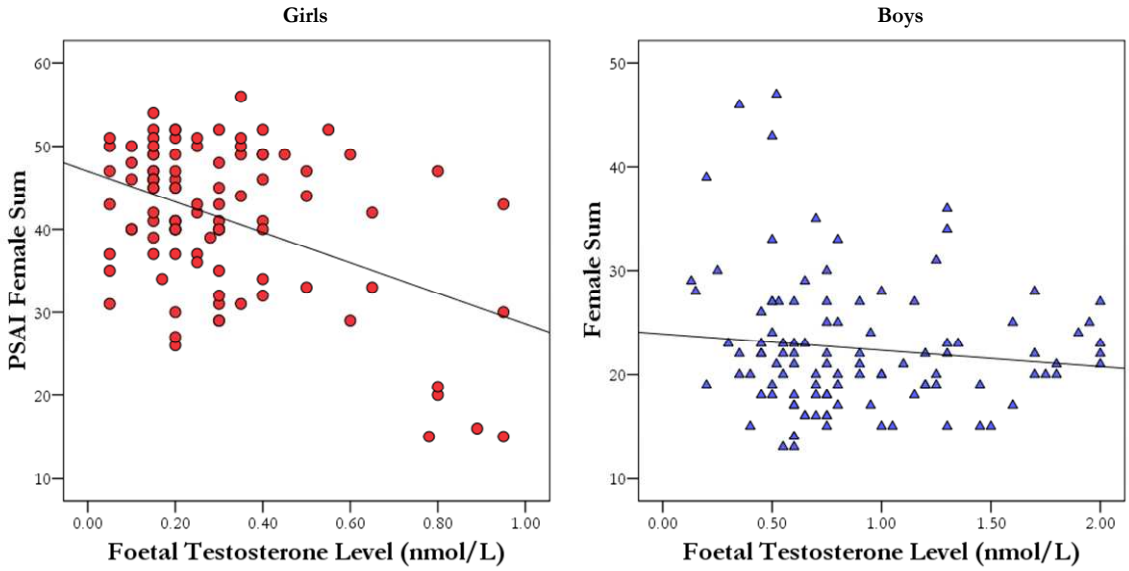


Table 3.7. Final regression model for PSAI Male sum

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
Male Sum	Gestational age	0.04	0.04	0.71	0.39	0.11	p>0.05
	Parent education			0.12	0.57	0.01	p>0.05
	Sex	0.45	0.41	4.22	0.73	0.47	p<0.001
	FT level	0.50	0.05	6.11	1.67	0.29	p<0.001
		Girls only					
Male Sum	Child age	0.06	0.06	1.15	0.65	0.18	p>0.05
	Parent education			1.70	0.83	0.21	p<0.05
	FT level	0.13	0.07	8.62	3.27	0.27	p<0.05
		Boys only					
Male Sum	Gestational age	0.06	0.06	0.59	0.58	0.12	p>0.05
	Older sisters			1.43	2.53	0.07	p>0.05
	Older brother			3.90	2.83	0.16	p>0.05

Table 3.7 shows the final regression models for PSAI Male sum. For boys and girls together, gestational age (r=-0.16, p<0.20) and mother age (r=0.14, p<0.20) were entered into the hierarchical multiple regression analysis in the first stage. The final regression model retained Sex and FT level (F-change=13.35, p<0.001, ΔR^2=0.05). The Sex/FT interaction was excluded. See Figure 3.6 for the relationship between FT levels and PSAI Male sum for boys and girls separately.

Figure 3.6. FT level and PSAI Male sum



A significant positive relationship between FT level and PSAI male sum scores was observed for both girls (r=0.23, p<0.05) and boys (r=0.23, p<0.05). For girls, child age (r=-0.14, p<0.20) and parent education level (r=0.18, p<0.20) were entered in the first stage. The final regression model included FT level in the second stage (F-change=4.34, p<0.01, ΔR^2=0.07). For boys, gestational age (r=-0.17, p<0.20) and presence of older brothers (r=0.20, p<0.20) were entered into the regression analysis. Presence of older sisters was entered as a suppressor variable. The regression analysis excluded FT level in the final model.

3.3.2. *BRSI Scores*

Examination of the distributions of the scales of the BSRI (M and F) were not skewed (skewness < 1), therefore raw F and M scores were used in subsequent analyses.

3.3.2.1. BSRI Internal Consistency and reliability

Cronbach's α coefficients were calculated and for the measure as a whole for the sexes combined (α=0.86), for girls (α=0.86) and for boys (α=0.87). The internal consistency of the BSRI F scale was satisfactory for the sexes combined (α=0.75), for girls (α=0.81) and for boys (α=0.72). The internal consistency for BSRI M scale was acceptable when the sexes were combined α=0.83, for girls (α=0.82) and for boys (α=0.88).

Split-half reliability was calculated for the measure as a whole. For the sexes combined, this was 0.87, for girls 0.83 and for boys 0.87.

3.3.2.2. BSRI F Score

Examination of F scores on the showed that girls (M=4.86, SD=0.66) had significantly higher scores than boys (M=4.50, SD=0.67), $t(205)=3.92$, $p<0.001$ (equal variances assumed). Figure 3.7 shows the distribution of BSRI F scores.

Figure 3.7. Distribution of F scores

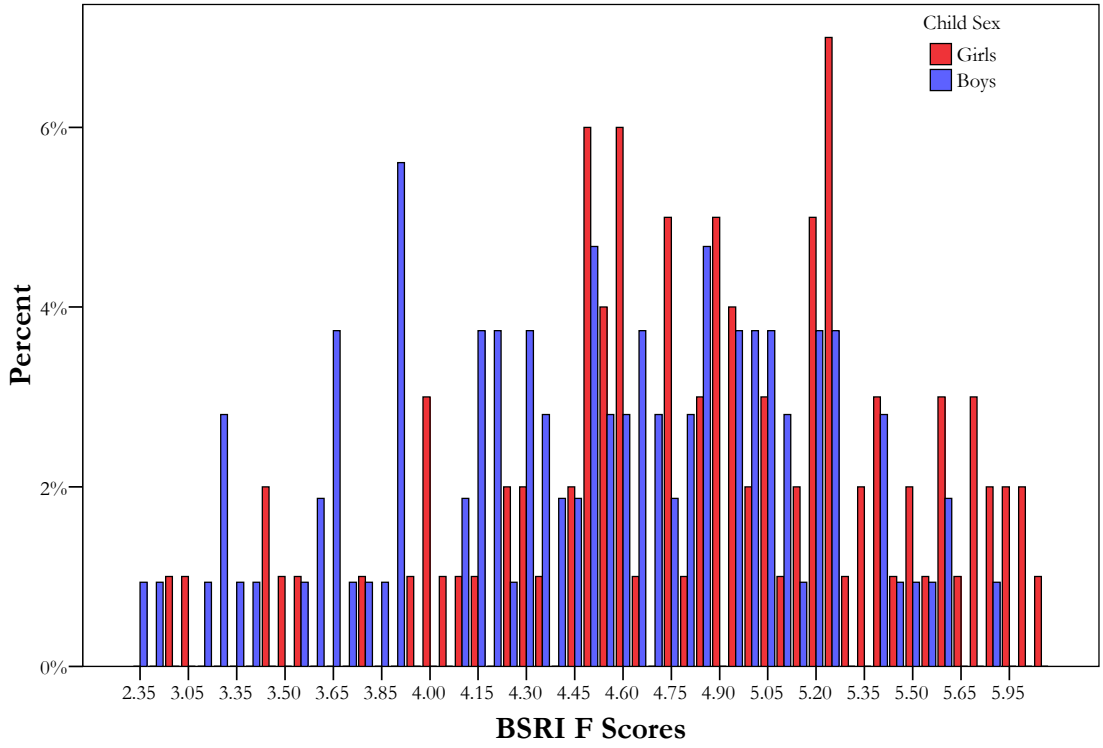


Table 3.8. Final regression model for F scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
F Score	No significant predictors						
		Girls only					
F Score	No significant predictors						
		Boys only					
F Score	Gestational age	0.18	0.18	0.09	0.06	0.19	p>0.05
	Child age			0.05	0.09	0.07	p>0.05
	Parent education			0.14	0.07	0.22	p>0.05
	Older sister			0.60	0.24	0.29	p<0.05
	Older brother			0.02	0.27	0.01	p>0.05

FT level did not show a significant correlation with F scores for boys and girls together (r=-0.05, p>0.05), or when girls were examined alone (r=-0.02, p>0.05) therefore

regression analyses were not conducted. Within sex analyses showed that F scores showed a significant correlation with FT level in boys ($r=0.23$, $p<0.05$), and regression analyses were conducted for F scores in this group. Gestational age ($r=0.17$, $p<0.20$), child age ($r=-0.15$, $p<0.20$), parent education ($r=-0.34$, $p<0.20$), presence of older sisters ($r=0.24$, $p<0.20$) and presence of older brothers (suppressor) were included in the first stage of the regression analysis. FT level was tested for entry in the second stage and was not retained in the final regression model.

3.3.2.3. BSRI M Score

Scores on the M scale showed significant sex-differences, $t(198.47)=2.60$, $p=0.01$ (equal variances not assumed), with boys ($M=4.65$, $SD=0.89$) scoring higher than girls ($M=4.37$, $SD=0.07$). See Figure 3.8 for the raw distribution of the M subscale scores.

Figure 3.8. Distribution of M scores

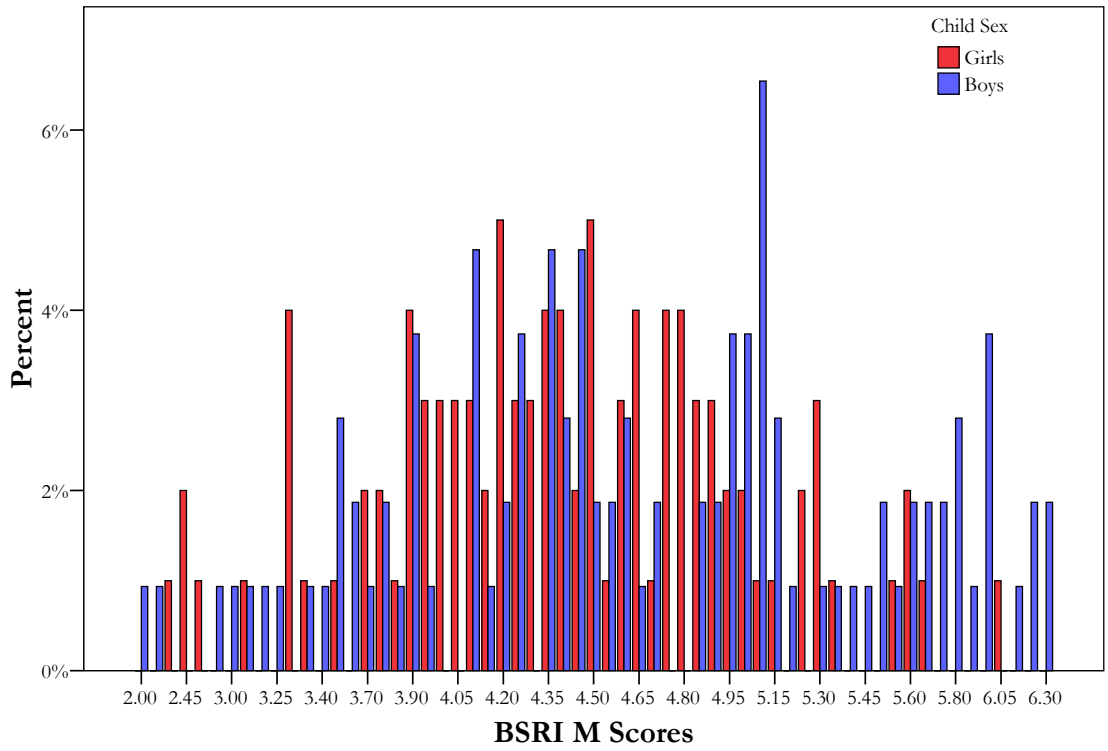
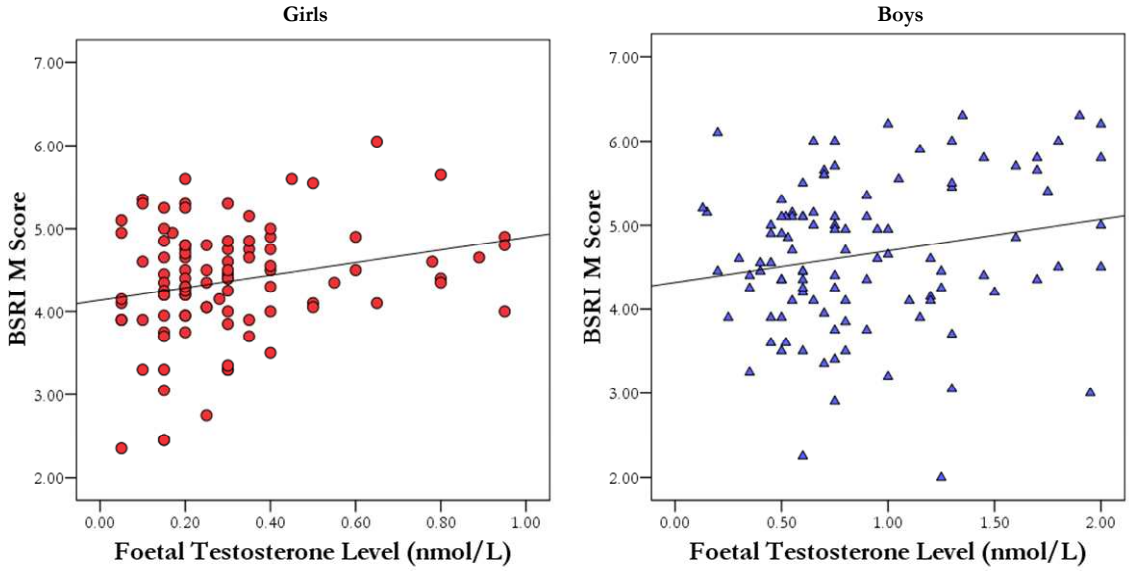


Table 3.9. Final regression model for M scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
M Score	FT level	0.07	0.07	0.46	0.12	0.27	p<0.001
		Girls only					
M Score	Gestational age	0.01	0.01	0.69	0.56	0.15	p>0.05
	FT level	0.10	0.09	10.60	4.18	0.30	p<0.05
		Boys only					
M Score	Child age	0.02	0.02	0.14	0.09	0.15	p>0.05

The predictor variables that correlated with M scores at $p<0.20$ were FT level ($r=0.27$, $p<0.001$) and sex ($r=0.18$, $p<0.05$), and these variables were included in the first stage of the regression analysis using the enter method. The only variable included in the final model was FT level ($F=15.91$, $p<0.001$, $R^2=0.07$). Within sex analyses were also conducted. For girls, gestational age ($r=-0.16$, $p<0.20$) was entered in the first stage and FT level ($r=0.24$, $p<0.05$) was tested for entry into the regression model in the second stage. The final regression model retained FT level ($F=3.59$, $p=0.03$, $\Delta R^2=0.09$). The only predictor variable that correlated with M score for boys was child age ($r=0.25$, $p<0.05$). FT level ($r=0.20$, $p<0.05$) was tested for entry in the second stage. Child age was the only variable retained in the final regression model for boys. See Figure 3.9 for the relationship between FT levels and M scores for girls and boys separately.

Figure 3.9. FT level and M scores



3.4. Discussion

The current study shows that FT levels significantly predict gender-typical play, measured using the Pre-School Activities Inventory (PSAI) when girls and boys are examined together and in girls when examined alone. These patterns were consistent when examining overall PSAI score, PSAI Female Sum and PSAI Male Sum. In addition, because children in the current study were developing typically, and because measures of testosterone were taken directly from the foetal environment, they provide evidence that prenatal testosterone plays a role in sexual differentiation of human behaviour.

Examination of Bem Sex Role Inventory (BSRI) scores showed that higher FT levels were associated with higher masculinity scores on the BSRI when boys and girls were examined together, and when girls were examined alone. No relationships were found between FT levels and scores on the femininity scale. Within sex results suggest that girls exposed to higher testosterone levels in utero are perceived as exhibiting more masculinised behaviour. This interpretation is consistent with other findings showing that lower (more masculine) 2D:4D ratio is related to BSRI scores (Csatho et al., 2003).

Furthermore, women with CAH (causing elevated prenatal androgen levels) usually differ from unaffected controls on scales of personality inventories by scoring in a more masculine direction (Collaer & Hines, 1995).

However, there was a significant correlation between each of the PSAI and BSRI scales and child sex. These results might suggest that females are particularly sensitive to changes in FT level, so that within sex results are only seen in girls. Results might also be affected by the timing of measurements relative to sensitive periods for development. Findings in primates show that there may be different sensitive periods for different behaviours (Goy, Bercovitch & McBrair, 1988). For example, studies of female rhesus macaques exposed to androgen early in gestation (and thus with virilised genitalia) show increased mounting behaviour, whereas those exposed late in gestation (with no genital virilisation) show increased rough play (Goy et al., 1988). There also may be an early postnatal sensitive period (Months 1 to 5; Smail et al., 1981), so that some of the behavioural masculinisation seen in typical boys and CAH girls may result from their continued exposure to androgen in the early neonatal period (Henderson & Berenbaum, 1997; Hines, 2004).

The measures used in this study tend to support a link between FT levels and gender-typical behaviour. However for both measures, results are less clear for boys. This might be because these gender-typical measures are designed to detect normal variation between males and females. As a result, these instruments may be less sensitive to within sex variation in males or variation between males and extreme male behaviour.

Other studies have also reported significant relationships in females. Hines et al. (2002a) reported a link between maternal testosterone levels and male-typical behaviour but only in girls. These results should be interpreted with caution since van de Beek et al. (2008) reported that maternal and foetal testosterone levels were not related to each other or to gender-typical play. However, this study used observational measures during a structured play session, and reported a low sex difference effect size for this measure. In

addition, the sample size was smaller than the present study and involved different measures of play preference.

Together with previous results, the present findings appear to show a significant relationship between prenatal exposure to testosterone and child gender-typical behaviour. Measurements also suggest that within sex variation of behaviour is associated with FT levels in girls. These results provide support for the idea that masculinisation of the brain might be affected by higher levels of FT. It is important to note that mothers taking part in this study were typically above average in age and education, thus restricting generalisability.

Nevertheless, the significant relationship in girls observed on both measures of gender-role behaviour does suggest that FT levels are involved in shaping these behaviours. These results are also in line with the finding that girls with ASC show a more masculinised play style, and that boys with ASC also show a play preference consistent with their sex (Knickmeyer et al., 2007). Therefore, studies relating amniotic fluid testosterone to subsequent behaviour may be useful for elucidating the role of prenatal hormonal exposure in gender-typical behavioural development.

Chapter 4: Foetal testosterone and aggression

In the area of aggression, consistent behavioural sex differences are found in humans. In this chapter, the relationship between FT and scores on two measures of aggressive behaviour in n=235 children are examined. No sex differences were observed for either of these measures. In addition, no relationships were found between FT levels and parent-reported aggression. Results from the current study suggest that it is likely that there are multiple determinants of the development of aggression. A study using direct measures of hormone levels as well as multiple measures of aggression, including parent, teacher and peer report in conjunction with observational measures are needed.

4.1. Introduction

In humans, one of the most consistent behavioural sex differences is observed in the area of aggression, with males more likely to exhibit this behaviour than females (Berenbaum & Resnick, 1997; Collaer & Hines, 1995; Hines, 2004; Hyde, 1984). Results from a meta-analysis by Hyde (1984) suggest that the sex difference in physical aggression in children is medium in size (d=0.58), and smaller in studies with college students (d=0.27). Observational studies have shown that sex differences are apparent in physical aggression from 2 years of age or younger (Archer, 2004), suggesting the possibility of a biological origin. The observed sex differences and early occurrence of these behaviours suggest that aggression may be influenced by sex hormones.

The investigation of aggression in humans is complex because of the limitations associated with the direct investigation and measurement of aggressive behaviour. Physical aggression is generally not approved of, making it more difficult to observe and measure than other characteristics (Hines, 2004). Methodological differences add to these difficulties, giving rise to the observation that sex differences observed in aggressive behaviour are larger in observational studies than in controlled experiments. Sex differences are also larger when assessment involves direct observation, peer report or projective tests (where the participant is asked to predict how they might react to situations), compared to self-report or reports from parents or teachers (Hyde, 1984). In addition, studies in laboratory settings typically rely on provoking aggressive behaviour. These difficulties in obtaining data on aggressive behaviour may help explain why relatively little is known about hormonal (or other) influences on aggression (Hines, 2004).

Studies examining the affects of hormones on aggression have typically relied on abnormal foetal environments rather than direct measurement. Reinisch (1981) examined the relationship between aggression and exposure to synthetic progestins (administered to pregnant women at risk for miscarriage). This study used a self-report

measure asking the participant to estimate their response in a variety of common conflict situations. Both males and females who were exposed to synthetic progestins had higher scores for physical aggression (but not verbal aggression) compared to unaffected siblings (Reinisch, 1981).

Cohen-Bendahan et al. (2005) examined aggressive behaviour in same-sex and opposite-sex twins, with the assumption that opposite-sex twin girls are exposed to higher levels of prenatal testosterone compared to same-sex twin girls. The researchers hoped to control for postnatal environmental effects by comparing data with similar measurements of same-sex female twins. In this study, the Dutch translation of the Reinisch Aggression Inventory (RAI) (Reinisch & Sanders, 1986) and the Dutch translation of a modified version of the Olweus Multifaceted Aggression Inventory (OMAI) (Finkelstein et al., 1997) were used to measure aggression in 74 opposite-sex and 55 same-sex 13-year-old twin pairs. Opposite-sex twin girls scored in the masculine direction on the Withdrawal and Verbal aggression subscales of the RAI, whereas no differences were observed between same and opposite-sex twin girls on the OMAI. These differences may have existed because the RAI measures how prone an individual is to aggressive behaviour, whereas the OMAI focuses on overt aggressive behaviour (Cohen-Bendahan et al., 2005b). Activational effects of testosterone were also investigated in this study by using salivary testosterone measures in addition to a measure of pubertal status. This was assessed using the Tanner drawings of pubertal development (Tanner, 1962). Although there was some evidence of associations between free testosterone levels and personality traits (such as aggressive impulses and boredom susceptibility in boys, and experience seeking and extraversion in girls), the authors concluded that at this age, no clear associations between circulating testosterone levels and behavioural traits were apparent (Cohen-Bendahan et al., 2005b).

Other evidence for a relationship between prenatal testosterone exposure and aggression has primarily come from studies of individuals with Congenital Adrenal Hyperplasia (CAH), which have shown inconsistent results (see Table 4.1). Two of these

Table 4.1. Studies of atypical androgen exposure and childhood aggressive behaviour

Study	Assessment Method	Measure	Group (Controls)	N's, probands (controls)	Aggressive Behaviour Outcome	
					Females	Males
Reinisch 1981	Self-report	Leifer-Roberts Response Hierarchy	Prenatal exposure to synthetic progestins (sibling controls)	17 F, 8 M (17 F, 8M)	Exposed females > control	Exposed males > control
Cohen-Bendahan et al. (2005)	Self-report	OMAI[a]	OS[b] (SS[c])	74 F (55 F)	ns	—
Cohen-Bendahan et al. (2005)	Self-report	RAI[c]	OS[b] (SS[c])	74 F (55 F)	OS[b] > SS[c]	—
Berenbaum & Resnick (1997) Sample 1	Self-report	MPQ[e] aggression subscale	CAH (sibling controls)	18 F, 11 M (13 F, 5 M)	CAH>control*	ns
Berenbaum & Resnick (1997) Sample 2	Self-report	MPQ[e] aggression subscale	CAH (sibling controls)	11 F, 17 M (5 F, 10 M)	ns	ns
Berenbaum & Resnick (1997) Sample 2	Retrospective self-report	RAI[d]	CAH (sibling controls)	11 F, 17 M (5 F, 10 M)	CAH>control**	ns
Berenbaum & Resnick (1997) Sample 3	Parent-report	RAI[d]	CAH (sibling controls)	20 F, 15 M (10 F, 20 M)	ns	ns
Ehrhardt and Baker (1974)	Self- and parent report	Semi-structured interview	CAH (sibling controls)	17 F, 10 M (11 F, 16 M)	CAH>control , ns	ns
Ehrhardt et al. (1968)	Self-report	Semi-structured interview	CAH (matched controls)	15 F (15 F)	ns	—
Money and Schwartz (1976)	Retrospective self- and parent report	Interview	CAH girls (—)	15 F	ns[g]	—
Pasterski et al. (2007)	Parent-report	ALEQ[f]	CAH (sibling controls)	38F, 29M (25F, 21M)	CAH>control**	ns

* $p<0.05$, ** $p<0.01$

[a] OMAI— Dutch translation of a modified version of the Olweus Multifaceted Aggression Inventory (OMAI) (Finkelstein et al., 1997). Participants are asked to answer questions for the following six scales: Physical Aggression Against Adults and Peers; Verbal Aggression Against Adults, and Verbal Aggression against Peers; Aggressive Inhibitory Responses; and Aggressive Impulses.

[b] OS—Opposite-sex female twins

[c] SS—Same-sex female twins

[d] RAI—Reinisch Aggression Inventory (Reinisch, 1981). This measure assesses potential for aggressive behaviour in hypothetical conflict situations.

[e] MPQ—Multidimensional Personality Questionnaire (Tellegen, 1982). Participants are asked to rate how aggressive, vindictive, or revengeful they are.

[f] ALEQ—Activity Level/Extraversion Questionnaire (Zucker and Bradley, 1995). This measure asks parents to rate how similar their child's behaviour is to the behaviour described.
[g] No control group was employed and no statistics were reported; authors concluded that there was no effect on aggressive behaviour given the low prevalence in their sample.
— Indicates that this group was not included in the study.
Table modified from: Pasterski, V., Hindmarsh, P., Geffner, M., Brook, C., Brain, C., & Hines, M. (2007). Increased aggression and activity level in 3- to 11-year-old girls with congenital adrenal hyperplasia (CAH). *Hormones and Behavior*, *52*, 368-374.

studies showed increased aggression in females (but not males) with CAH using both self-report (Berenbaum & Resnick, 1997) and parental-report measures (Pasterski et al., 2007). Findings from a much earlier, interview-based study of children with CAH suggested a similar trend but the results were not statistically significant (Ehrhardt & Baker, 1974). However, two studies found no relationships between children with CAH and aggressive behaviour (Ehrhardt, Epstein & Money, 1968; Money & Scwartz, 1976). Several factors such as sample sizes, use of retrospective measures, lack of control samples and possible illness effects associated with this condition may have contributed to these mixed results. Therefore, although some evidence from females with CAH suggests that prenatal exposure to high levels of androgenic hormones may be associated with physical aggression, findings are not conclusive.

Further evidence examining the relationship between prenatal hormone exposure and aggression has come from studies of 2D:4D ratio. One study examined indirect and direct aggression in a sample of 100 female university students (Coyne et al., 2007). No relationships were found between digit ratio and aggression, measured using the Aggression Questionnaire (Buss & Perry, 1992). These data are consistent with observations made by Austin et al. (2007) who found no associations between digit ratio and aggression using the same measure for men or women (Austin et al., 2002). However, Coyne et al. (2007) found directional asymmetry (left digit ratio minus right digit ratio) in women to be related to indirect aggression, measured using the Indirect Aggression Questionnaire (Forrest, Eatough & Shevlin, 2005). Women who had low directional asymmetry showed more indirect aggression, suggesting a positive association between prenatal testosterone exposure and indirect aggression in women (Coyne et al., 2007). Another study using Buss and Perry's (1992) Aggression Questionnaire found an association between digit ratio and aggression in men but not women (Bailey & Hurd, 2005). In addition, an investigation also using the Aggression Questionnaire (Buss & Perry, 1992) found significant associations with digit ratio in the sample as a whole and in women (Hampson et al., 2008). Finally, one study using a modified version of a competitive reaction-time task used to elicit and measure

aggression showed a correlation between masculinised (lower) digit ratio and aggressive behaviour in males but not females (Kuepper & Hennig, 2007). Thus, measurements using 2D:4D ratios have generally supported a role for prenatal testosterone exposure in the development of aggressive behaviour but the results are not entirely consistent.

Autism Spectrum Conditions (ASC) are made up of a number of conditions, which are frequently accompanied by challenging behaviour (Matson, Dixon & Matson, 2005; Matson & Nebel-Schwalm, 2007). A recent meta-analysis showed that individuals with autism were significantly more likely to show aggression, self-injury and disruption to the environment (McClintock, Hall & Oliver, 2003). However, despite the general consensus that these behaviours frequently occur in developmental disabilities and in ASC in particular, little is known about why these behaviours arise or whether there is any link to prenatal hormone exposure. This is perhaps because these behaviours are rarely screened for or included as outcome measures in research studies of young individuals with ASC.

4.1.1. *Aims*

The current study aims to directly investigate the relationship between aggressive behaviour and FT levels measured in the second trimester of pregnancy. This is the first study to examine parent-reported aggressive behaviour and associations with a direct, quantitative measure of prenatal testosterone exposure.

4.2. Methods

4.2.1. *Participants*

The Child Behaviour Checklist-Aggression Subscale (CBC-A) and Children's Aggression Scale (CAS) were sent to all the mothers from the Cambridge FT cohort (n=456), resulting in 235 (119 girls, 116 boys) children with complete data.

4.2.2. *Outcome variables*

Child Behaviour Checklist-Aggression Subscale (CBC-A) (Achenbach & Edelbrock, 1983). The CBC-A obtains reports from parents, other close relatives, or guardians regarding children's competencies and behavioural/emotional problems (Achenbach & Edelbrock, 1983). The CBC-A has 19 items that describe specific behaviours, and an open-ended item for reporting additional problems. Parents rate how true each item is now or within the past 6 months using the following scale: 0=not true; 1=somewhat or sometimes true; 2=very true or often true. The Child Behaviour Checklist is a widely used measure that has been developed and tested in large population samples. Due to the large number of items (118 items) included in the Child Behaviour Checklist, only the 20 items making up the aggression subscale were administered to parents. See Appendix 3.

Children's Aggression Scale-Parent Version (CAS-P) (Halperin, McKay & Newcorn, 2002). The CAS-P was designed to evaluate the frequency and severity of aggressive acts, as distinct from oppositional and defiant behaviours, in children. The scale has 33 items representing five domains: Verbal Aggression, Aggression Against Objects and Animals, Provoked Physical Aggression, Unprovoked Physical Aggression, and Use of Weapons. The weapons section was excluded since it was unlikely that this behaviour would be observed in this sample of children. This portion of the questionnaire had a total of 28 items, and the parent rates how often their child exhibits a behaviour using the following scale: 0=Never; 1=Once a month or less; 2=Once a week or less; 3=2-3 times a week; 4=Most Days. See Appendix 4.

4.2.3. *Predictor variables*

Chapter 2 provides a detailed description of the predictor variables utilised in this study. FT level was the predictor of greatest interest. The control variables that were included in the subsequent analyses were gestational age at amniocentesis, maternal age, level of education obtained by the parents, presence of older siblings and child's age.

4.3. Results

Inspection of the univariate distributions revealed that foetal testosterone level was positively skewed, and was the only predictor variable with a distribution that deviated significantly from the Gaussian distribution. Three female outliers in FT levels (individuals who scored three or more standard deviations from the mean) were observed. These outlying values were replaced using a windsorizing procedure, where the extreme values are replaced by the highest observed level within three standard deviations from the mean (0.95 nmol/L). No outliers were found when boys' FT levels were examined. Windsorized FT levels showed no outliers and acceptable skewness statistics for both boys and girls, and are used in subsequent analyses.

Table 4.2 presents the means and standard deviations for each sex separately, as well as combined for predictor and outcome variables.

Table 4.3 shows the correlation coefficients for predictor and outcome variables for all cases. Tables 4.4 and 4.5 show correlation coefficients for girls and boys separately.

Table 4.2. Descriptive statistics

	Combined Group				Girls				Boys				
Variable	n	M	SD	Range	n	M	SD	Range	n	M	SD	range	Cohen's d
^FT level (nmol/L)**	235	0.58	0.43	0.05-2.05	119	0.33	0.27	0.05-1.75	116	0.82	0.42	0.05-2.05	1.39
Gestational Age	161	16.51	1.46	13-22	80	16.65	1.49	14-22	81	16.37	1.42	13-20	0.19
Child Age	210	10.26	11.74	6.97-10.68	108	8.73	0.96	7.01-10.67	102	8.98	0.86	6.97-10.68	0.27
Maternal Age	200	41.32	4.40	29.42-53.23	101	41.26	4.31	29.42-53.13	99	41.38	4.51	31.68-53.23	0.03
Parental Education	199	3.24	1.01	1-5	101	3.17	0.87	1.5-5	98	3.32	1.13	1-5	0.15
^CBC-A Total	235	5.72	5.23	0-26	119	5.13	4.65	0-26	116	6.33	5.73	0-26	0.23
^CAS Total	235	11.28	10.74	0-62	119	9.98	9.84	0-54	116	12.60	11.48	0-62	0.25
^Verbal Total	235	8.34	7.69	0-47	119	7.55	7.01	0-37	116	9.15	8.28	0-47	0.21
^Objects Total	235	1.03	1.12	0-6	119	0.86	0.92	0-5	116	1.22	1.28	0-6	0.32
^Physical Total**	235	1.41	1.81	0-8	119	1.13	1.71	0-8	116	1.70	1.88	0-7	0.32
^Initiated Physical	235	0.49	1.15	0-9	119	0.45	1.14	0-9	116	0.54	1.15	0-6	0.08

^Indicates raw values

* Sex difference significant at the $p<0.05$ level

** Sex difference significant at the $p<0.01$ level

Table 4.3. Correlation matrix for all cases

	FT Level	Sex	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	CBC	CAS	Verbal	Object	Physical
Sex	.61**	--	--	--	--	--	--	--	--	--	--	--	--
Gestational Age	-.06	-.10	--	--	--	--	--	--	--	--	--	--	--
Child Age	.02	-.11	.08	--	--	--	--	--	--	--	--	--	--
Maternal Age	.07	.01	-.33	-.03	--	--	--	--	--	--	--	--	--
Parent Education	.08	.07	-.12	.11	.12	--	--	--	--	--	--	--	--
Older Sister	-.04	-.01	-.08	-.05	-.08	-.07	--	--	--	--	--	--	--
Older Brother	-.07	-.09	-.01	-.04	-.03	-.15*	.32**	--	--	--	--	--	--
CBC-A Total	.05	.10	-.14	-.08	.01	-.01	-.01	-.06	--	--	--	--	--
CAS-P Total	.05	.13	-.17*	-.08	-.11	.10	-.05	.04	.46**	--	--	--	--
Verbal Total	.04	.11	-.16*	-.07	-.12	.10	-.05	.04	.46**	.99**	--	--	--
Objects Total	.04	.16*	-.19*	-.14*	.01	.06	.05	.01	.45**	.68**	.63**	--	--
Physical Total	.10	.18**	-.15	-.05	-.07	.09	-.04	.07	.28**	.82**	.76**	.46**	--
Initiated Physical	.03	.15	-.09	.06	.07	.12	-.17	-.21	.34*	.70**	.59**	.38**	.61**

* $p<0.05$, ** $p<0.01$

Note: Values shown are from transformed data

Table 4.4. Correlation matrix for Girls

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	CBC	CAS	Verbal	Object	Physical
Gestational Age	.04	--	--	--	--	--	--	--	--	--	--	--
Child Age	.06	.10	--	--	--	--	--	--	--	--	--	--
Maternal Age	.11	-.34**	-.05	--	--	--	--	--	--	--	--	--
Parent Education	.04	.02	.21*	.07	--	--	--	--	--	--	--	--
Older Sister	-.05	.05	-.06	-.16	-.16	--	--	--	--	--	--	--
Older Brother	-.14	-.01	-.06	-.07	-.23*	.33**	--	--	--	--	--	--
CBC-A Total	-.09	-.23	-.09	.06	-.04	-.11	-.13	--	--	--	--	--
CAS-P Total	-.10	-.24*	-.09	-.12	-.03	-.04	-.01	.50**	--	--	--	--
Verbal Total	-.10	-.24*	-.09	-.12	-.04	-.04	-.01	.51**	.99**	--	--	--
Objects Total	-.01	-.11	-.19	.05	.00	.04	-.02	.41**	.65**	.61**	--	--
Physical Total	-.12	-.16	-.04	-.05	.05	-.05	.02	.27**	.77**	.72**	.31**	--
Initiated Physical	-.06	-.12	-.10	.03	.24	-.18	-.30	.38*	.66**	.51**	.45*	.63**

* $p<0.05$, ** $p<0.01$

Note: Values shown are from transformed data

Table 4.5. Correlation matrix for Boys

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	CBC	CAS	Verbal	Object	Physical
Gestational Age	-.02	--	--	--	--	--	--	--	--	--	--	--
Child Age	-.15	.06	--	--	--	--	--	--	--	--	--	--
Maternal Age	-.03	-.31**	.10	--	--	--	--	--	--	--	--	--
Parent Education	.04	-.21	-.13	.16	--	--	--	--	--	--	--	--
Older Sister	-.04	-.19	-.19	.00	.01	--	--	--	--	--	--	--
Older Brother	.07	-.02	.06	.03	-.05	.30**	--	--	--	--	--	--
CBC-A Total	.01	-.06	-.12	-.04	.00	-.09	.05	--	--	--	--	--
CAS-P Total	-.01	-.09	.12	-.11	.18	-.05	.13	.41**	--	--	--	--
Verbal Total	.00	-.07	.11	-.12	.19	-.05	.13	.41**	.99**	--	--	--
Objects Total	-.11	-.24*	.13	-.03	.09	.06	.09	.47**	.70**	.64**	--	--
Physical Total	.03	-.12	.07	-.10	.08	-.03	.17	.26**	.85**	.79**	.57**	--
Initiated Physical	.03	-.09	.06	.02	-.01	-.12	-.09	.36	.76**	.70**	.29	.59**

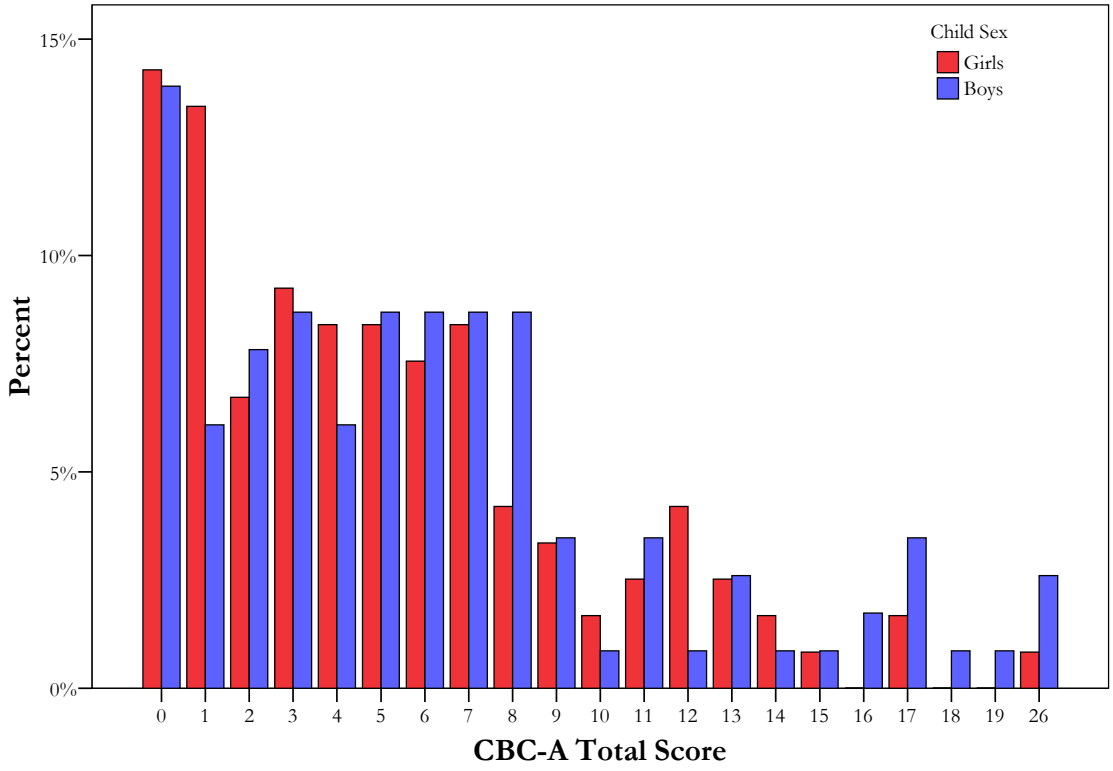
* $p<0.05$, ** $p<0.01$

Note: Values shown are from transformed data

4.3.1. *Child Behaviour Checklist-Aggression Subscale Scores (CBC-A)*

The distribution of CBC-A scores was positively skewed and a square-root transformation was applied. Transformed data are used for all subsequent analyses. See Figure 4.1 for the raw distribution of CBC-A raw scores.

Figure 4.1. Distribution of raw CBC-A Total Score



4.3.1.1. Internal Consistency

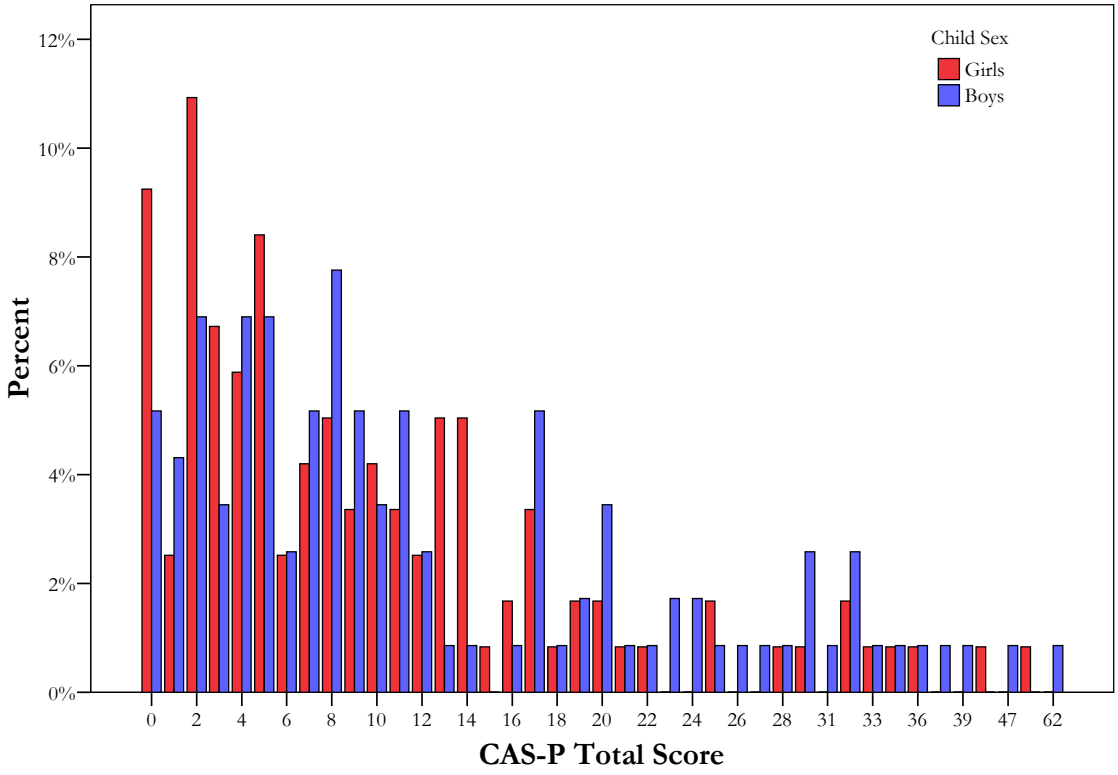
Cronbach's α coefficients were calculated and for the measure as a whole, indicating high internal consistency (α=0.88). The internal consistency for the entire measure was also high for girls (α=0.86) and boys (α=0.89) separately. Split-half reliability was acceptable (0.76) for the sample as a whole, for girls (0.79) and for boys (0.73).

No significant sex-differences were found for CBC-A scores, t(233)=1.64, p>0.05 between boys (M=2.51, SD=1.02) and girls (M=2.30, SD=0.92). No significant correlations between CBC-A and predictor variables were found (all p>0.05), therefore regression analyses were not conducted for these variables.

4.3.2. *Children's Aggression Scale-Parent Version (CAS-P).*

Examination of the distributions of the CAS-P total and subscale scores showed that they were positively skewed. A square-root transformation was carried out on these variables, yielding scores that were not skewed. Figure 4.2 shows the raw distribution of scores.

Figure 4.2. Distribution of raw CAS-P Total Score



4.3.2.1. Internal Consistency

Cronbach's α coefficients for CAS-P total score was high for the boys (α=0.86) and girls (α=0.85) separately and combined (α=0.86). Split half reliability for the measure as

a whole was acceptable for boys and girls together (0.77), and in boys (0.79) and girls (0.73) alone.

The internal consistency of the four CAS-P subscales was varied (see Table 4.7).

Table 4.6. Internal consistency of the CAS-P subscales

Scale	Sexes combined	Girls	Boys
CAS-P Total	.86	.85	.86
Verbal Aggression	.80	.78	.82
Aggression Against Objects and Animals	.35	.25	.39
Provoked Physical Aggression	.52	.53	.50
Initiated Physical Aggression	.58	.57	.59

Note: Internal consistency was calculated using Cronbach's α

No significant sex-differences were found between boys (M=3.38, SD=1.48) and girls (M=3.02, SD=1.37) for CAS-P total scores, $t(233)=1.93$, $p>0.05$. For the CAS-P Verbal Aggression subscale, no sex differences were found, $t(233)=1.58$, $p>0.05$. For the Aggression Against Objects and Animals subscale, a trend towards boys (M=0.89, SD=0.66) showing higher scores than girls (M=0.69, SD=0.62) was observed, $t(233)=2.39$, $p=0.02$. However, using the Bonferoni correction, this difference was not significant. The Provoked Physical Aggression subscale did show significant sex differences with boys (M=1.55, SD=0.54) scoring higher than girls (M=1.37, SD=0.50), $t(233)=2.69$, $p<0.01$. Finally, the Initiated Physical Aggression subscale showed no significant sex differences $t(233)=0.70$, $p>0.05$.

Examination of correlation coefficients revealed no associations between CAS-P scores and the predictor variables (all $p>0.05$), therefore regression analyses were not conducted for these variables.

4.4. Discussion

The aim of the current study was to examine if FT levels were related to aggression, measured by the Child Behaviour Checklist-Aggression Subscale (CBC-A) and the Children's Aggression Scale-Parent Version (CAS-P). No significant associations between the outcome and predictors were observed. In addition, significant sex differences were only found for the Provoked Physical Aggression subscale of the CAS-P. If differences in sexually dimorphic behaviours are influenced by exposure to hormones, the lack of a link between FT and aggression might be accounted for by the lack of a sex difference in this behaviour.

In this study, both measures of aggression reported skewed results, with a bias towards low scores. It is possible that the measures used in this study did not reflect the normal range of aggressive behaviour observed in children, resulting in possible floor effects. For CBC-A total raw score, 24% of the participants had a score of 0 or 1. For CAS-P total raw score, 11% of the participants had a score of 0 or 1. The absence of sex differences of these measures may also reflect the possible floor effects of the measures.

The parent-report nature of this study may also have introduced bias into the results, with parents reluctant to identify their child as being particularly aggressive in this sample. Perhaps these relatively rare types of behaviour would be difficult to detect in a questionnaire study, since the response rate of the current study was 52%, and unknown factors may be contributing to the lack of response in a certain subset of these mothers. This is unfortunately a limitation that is difficult to eliminate when relying on voluntary responses from research participants. Conversely, other evidence suggests that parent-report may be a reliable method of characterising aggression (Meyer-Bahlburg, Erhardt & Feldman, 1985), and may also reduce social desirability bias associated with measures such as interviews (Richman et al., 1999). A strength of parent-report is that it typically allows the inclusion of a larger number of participants, increasing statistical power.

However, it is possible that some parents may underestimate their children's problem behaviours, and that social desirability may affect scores.

A recent review by Archer (2004) examined longitudinal studies of aggression showing that limited data suggests that sex differences in physical aggression were largest between the ages of 18 to 30 years of age. One possibility is that aggressive behaviour is influenced by the activational effects of circulating testosterone. Studies of adolescent males have shown that circulating testosterone levels were positively associated with self-report hypothetical measures of physical and verbal aggression (Olweus et al., 1980), supporting this hypothesis. The findings reported by Olweus et al. (1980) are consistent with meta-analytic results showing a small, positive correlation between aggressive behaviour and current testosterone levels (Archer, 1991; Book, Starzyk & Quinsey, 2001). However, a drawback of relying on meta-analyses is that the only studies that are included are those that have reported effect sizes and p-values, possibly underestimating the proportion of non-significant findings in the literature (Book et al., 2001).

Research examining the influence of family members and indirect aggression suggests that there are multiple factors which contribute to the development of interpersonal aggression (Williams, Conger & Blozis, 2007). Williams et al. (2007) suggest that factors such as gender and aggression of older siblings, rates of parental hostility and aggression in younger siblings predict the development of interpersonal aggression. The measurement of such variables was beyond the scope of this study. The limited data do, however, suggest that the development of aggression is not related to the presence of older siblings, maternal age or parental education. More direct measures of aggression such as naturalistic observation and multiple methods of assessment are needed to help clarify the factors involved in the development of aggressive behaviour.

McClintock et al. (2003) suggest that aggression is more common amongst males, in individuals with a diagnosis of autism and in individuals with a deficit in expressive communication. Despite the more frequent occurrence of aggressive behaviour in

children with ASC, most diagnosticians are of the opinion that challenging behaviours are not core features of ASC, and that these maladaptive responses co-vary with ASC at a high rate (Matson & Nebel-Schwalm, 2007). If high levels of FT are associated with the development of behaviours related to ASC, then this might help to explain the lack of relationships with FT levels and aggressive behaviour.

It is difficult to draw conclusions about the effect of prenatal hormones on aggression using the results of this study. The lack of a clear sex difference in the measures of aggression used indicates one possible explanation for the absence of a link to FT. The nature of aggression is complex with aspects of the interaction affecting the expression of this behaviour. Results from the current study suggest that it is likely that there are multiple determinants of the development of aggression. A study using direct measures of prenatal hormone exposure as well as multiple measures of aggression, including parent, teacher and peer report in conjunction with observational measures would need to be conducted before clear conclusions can be made.

Chapter 5: Foetal testosterone, empathising and systemising

In this chapter, the E-S theory of sex differences is investigated in a large sample of typically developing children and children with ASC. Study 1 reports the development of the children's versions of the Empathising Quotient (EQ-C) and Systemising Quotient (SQ-C). The EQ-C and SQ-C were administered to n=1256 parents of typically developing children aged 4-11 years. Both measures showed good test-retest reliability and high internal consistency for the empathising and systemising components. On the EQ-C, girls scored significantly higher. On the SQ-C, boys scored significantly higher than girls. A sample of children with ASC (n=265) scored significantly higher on the SQ-C, and significantly lower on the EQ-C, compared to typical boys. Empathising and systemising in children showed similar patterns of sex differences observed in adults. Children with ASC tended towards a 'hyper-masculinisation' profile, irrespective of sex. Study 2 investigated the relationship between FT and EQ-C and SQ-C scores in n=208 children. Results showed a significant negative correlation with EQ-C, but results from the regression analysis suggests that sex plays a larger role in predicting these scores than FT. For SQ-C scores, a significant positive relationship with FT was found. FT was the only significant predictor retained in the final regression model, suggesting that FT levels play a greater role than the child's sex in terms of differences in systemising preference.

5.1. Introduction

Baron-Cohen (2002) suggests that rather than the traditional sex differences examined in verbal and spatial ability, using the dimensions of 'empathising' and 'systemising' might also aid in the understanding of human sex differences. The Empathising-Systemising (E-S) theory proposes that these dimensions are central to sex differences in the mind: empathising (the drive to identify another person's emotions and thoughts, and to respond to these with an appropriate emotion) is held to be generally stronger in females, whilst systemising (the drive to analyse, explore and construct a system) is held to be generally stronger in males.

5.1.1. *Sex differences in empathising and systemising*

Sex differences in the precursors of empathy are seen from birth, with female babies showing a stronger preference for looking at social stimuli (faces) 24 hours after birth (Connellan et al., 2000), and more eye contact at 12 months of age (Lutchmaya et al., 2002a). Girls pass false belief tests earlier (Cutting & Dunn, 1999), and are better than boys at evaluating the feelings and intentions of characters in a story (Bosacki & Astington, 1999). A female superiority has also been observed on the 'faux pas' test (Baron-Cohen, 1999), which measures recognition of when a character says something that might hurt another character's feelings. Girls show better quality of social relationships at 48 months old, as measured on the Children's Communication Checklist (Knickmeyer et al., 2005a). Girls also tend to show more comforting, sad expressions or sympathetic vocalisations than boys when witnessing another's distress as early as one year of age (Chakrabarti & Baron-Cohen, 2006). Such sex differences in empathy remain evident in adulthood: for example, women score higher than men on the 'Reading the Mind in the Eyes' task (Baron-Cohen et al., 1997).

Studies have also found higher levels of competition and direct aggression in boys (Maccoby & Jacklin, 1974; Olweus et al., 1980). Competition and aggression arguably suggest lower empathy. Furthermore, boys show more 'rough-and-tumble play' than

girls, which might indicate lower empathy since it can be painful or intrusive (Maccoby, 1998).

Boys, on average, engage in more mechanical and constructional play than girls, demonstrated by the preference for boys to play with toy vehicles or Lego™ sets, while girls are more likely to choose to play with dolls or toy animals (Berenbaum & Hines, 1992; Liss, 1979). This sex difference in toy choice has been observed in humans as early as the first year of life (Servin et al., 1999), as well as in nonhuman primates (Alexander & Hines, 1994), suggesting a biological basis for these preferences. Boys are better than girls at using directional cues in map-reading and map-making (Beatty & Tröster, 1987; Galea & Kimura, 1993; Kimura, 1999). They are also more accurate on the Mental Rotation Test (Johnson & Meade, 1987; Masters & Sanders, 1993), and the Rod and Frame Test (Berlin & Languis, 1981; Witkin et al., 1962). All of these can be seen as involving systemising since they involve relating input to output via a lawful operation. Boys have also shown faster performance than girls on the Embedded Figures Test (EFT) (Berlin & Languis, 1981; Witkin et al., 1962). The EFT measures attention to detail and field independence (Nebot, 1988), which are considered to be components of systemising.

5.1.2. *The Adult EQ and SQ*

In order to explore the degree to which an individual empathises and systemises, researchers developed the Empathising (EQ) and Systemising Quotients (SQ) (Baron-Cohen et al., 2003; Baron-Cohen & Wheelwright, 2004). Both of these self-report questionnaires have a forced-choice format. The questionnaires also contain a list of statements about real life situations, experiences and interests, where systemising or empathising skills are required.

Initial findings using the EQ in adults revealed a significant sex difference, with women (M=48.0, SD=11.3) scoring higher than men (M=39.0, SD=11.6). Since its original

development, the SQ (Baron-Cohen et al., 2003) has been revised to include more gender-neutral activities (Wheelwright et al., 2006). Results indicate that men (M=61.2, SD=19.2) score higher than women (M=51.7, SD=19.2). EQ and SQ scores have been found to be better predictors of career choice in science and engineering, or in degree choice (science versus humanities) than sex (Billington, Baron- Cohen & Wheelwright, 2007; Focquaert et al., 2007), suggesting that typical sex differences in interests or aptitudes reflect the individual's cognitive 'brain type' rather than their sex.

Whilst an individual's ability in empathising or systemising are expected to vary, the clearest differences between the sexes were found in terms of the difference in standardised scores between the EQ and SQ. These were defined as follows:

$$E = (EQ_{Observed} - EQ_{GroupMean})/EQ_{MaxScore}$$

$$S = (SQ_{Observed} - SQ_{GroupMean})/SQ_{MaxScore}$$

Five cognitive profiles, called 'brain types', emerged in a general population of adults (Wheelwright et al., 2006):

(1) Type E is defined as E>S (empathising being at a higher level than systemising). Type E was found in 44.8% of females and 15.1% of males, and will be referred to as the typical 'female' brain type.

(2) Type S is defined as S>E, which is more common in males. Type S was found in 49.5% of males and 20.7% of females, and will be called the typical 'male' brain type.

(3) Type B (for balanced) is the profile where E=S. 30.3% of males and 29.3% of females had the profile of Type B.

(4) An extreme of Type S (S>>E) was seen in 5.0% of males and 0.9% of females.

(5) An extreme of Type E (E>>S) was seen in 4.3% of females and 0.1% of males. These can be thought of as the extreme male brain type and the extreme female brain type.

The Extreme Male Brain (EMB) theory of autism (Baron-Cohen, 2002; Baron-Cohen & Hammer, 1997) is an extension of the E-S model of sex differences. The EMB theory proposes that individuals with ASC are impaired in empathising whilst being at least average or superior in systemising.

5.1.3. *Hormones and the E-S theory*

Research directly examining the relationship between prenatal exposure to testosterone and the development of empathising and systemising is limited. Studies investigating the relationship between FT levels measured in amniotic fluid have shown a significant negative relationship with amount of eye contact in 12-month-olds when the sexes were combined and in boys (Lutchmaya et al., 2002a). In addition, the quality of social relationships in 4-year-olds has been found to be inversely related to levels of FT when the sexes were combined and in boys (Knickmeyer et al., 2005a). Eye contact and quality of social relationships are both sexually dimorphic areas shown to be stronger in girls, and these findings were taken to support a role for foetal testosterone in the development of behaviours related to empathy.

Indirect studies using 2D:4D ratios as a proxy measure for prenatal androgen exposure have provided little support for a significant role of prenatal hormonal effects on behaviours such as empathy. One study using the Emotional Empathy Scale (Mehrabian & Epstein, 1972) found no correlation between digit ratio and scores in a sample of n=162 adults when the effects of sex were controlled for (Hampson et al., 2008). Another study examined digit ratio and its relationship to measures of empathising and systemising in n=423 Austrian adults (Voracek & Dressler, 2006). No significant relationships were observed between digit ratio and measures of empathy using Baron-

Cohen et al.'s (2001) "Reading the Mind in the Eyes test", Adult EQ scores, Adult SQ scores or Autism Spectrum Quotient (AQ) scores. However, as discussed, caution should be exercised when using 2D:4D ratio as a proxy for prenatal androgen exposure, since individual differences in 2D:4D may be subject to genetic factors which could be more influential than the effects of common prenatal environmental factors (Knickmeyer et al., 2008; Paul et al., 2006).

5.1.4. *Aims*

There are two main objectives of the work described in this chapter. The aim of Study 1 is to develop child versions of the EQ and SQ (EQ-C and SQ-C), and to examine if the scoring patterns of these measures are consistent with the typical sex differences observed in adulthood. It is also predicted that children with ASC will score lower on the EQ-C and higher on the SQ-C compared to typically developing boys, as predicted by the EMB theory of autism. Study 2 aims to examine whether prenatal hormones (FT levels), measured from amniotic fluid, play a role in EQ-C and SQ-C scores, and to examine if FT levels are related to the cognitive brain type a child displays.

5.2. Study 1: Development of the EQ-C and SQ-C

5.2.1. *Study 1 Method*

5.2.1.1. Instrument Development

The EQ-C and SQ-C were adapted from the adult versions of the EQ and SQ, and are shown in Appendix 5. Items were worded to produce an approximately equal agree/disagree response in order to avoid a response bias. In this study the EQ-C and SQ-C were combined into one questionnaire for ease of administration. This new questionnaire was also designed to be based on parent-report, since self-report in children might be confounded by reading and comprehension abilities. The questionnaire was designed to be short, easy to use and self-administered. Items were

chosen that would be equally applicable to boys and girls. Items that were not age-appropriate from the adult questionnaires were eliminated, whereas other items were adapted to be age-appropriate.

5.2.1.2. Pilot study

A sample of 22 children (12 males, 10 females) ages 5 to 11 years (M=8.1, SD=1.79) were recruited for a pilot study. Ceiling and floor effects were absent, and a broad range of scores for empathising and systemising were obtained. Participants were given the opportunity to express any comments they had about the questionnaire. No revisions were found to be necessary.

5.2.1.3. Study 1 Participants

Questionnaires were completed by mothers of children, ages 4 to 11 years (M=7.90, SD=1.77). These fell into 2 groups:

Group 1 consisted of n=1256 (675 girls, 581 boys) typically developing children who were participating in a large epidemiological study of social and communication skills in primary school in and around Cambridge, UK (Scott et al., 2002b; Williams et al., 2005).

Group 2 consisted of n=265 children (46 girls, 219 boys) with a diagnosis of ASC. Mothers of children with ASC were recruited in several ways: local newspaper articles inviting mothers to participate in the research study were used in the Cambridge region and via the Autism Research Centre participant website, University of Cambridge (www.autismsresearchcentre.com). Mothers completed the questionnaires online. Information such as the date of diagnosis, organisation and clinician who administered the diagnosis was collected, and children with a diagnosis of Asperger syndrome, High-Functioning autism and autism were included in the study.

5.2.1.4. Scoring

The combined EQ-C and SQ-C is a 55 item parental-report forced-choice questionnaire, with four alternatives for each question. The parent indicates how strongly they agree with each statement about their child by ticking one of several options: 'definitely agree', 'slightly agree', 'slightly disagree' or 'definitely disagree'. The scoring of each item gives a value of 0, 1 or 2. A value of 2 indicates a definitely agree or disagree response (a strong empathising or systemising trait), a value of 1 indicates a slightly agree or disagree response (partial presence of the trait), and a value of 0 indicates the trait's absence. Questionnaires with five or more blank items were considered incomplete, and these data were discarded in subsequent analyses (n=7). The 55 items were split into 27 EQ-C questions and 28 SQ-C questions:

(a) For the EQ-C, a 'slightly agree' response scores one point and 'strongly agree' scores two points on the following items: 1, 6, 14, 18, 26, 28, 30, 31, 37, 42, 43, 45, 48 and 52. 'Slightly disagree' scores one point and 'strongly disagree' scores two points on the following items: 2, 4, 7, 9, 13, 17, 20, 23, 33, 36, 40, 53 and 55. The maximum attainable score for this domain is 54.

(b) For the SQ-C, a 'slightly agree' response scores one point and 'strongly agree' scores two points on the following items: 5, 8, 10, 12, 19, 21, 24, 25, 29, 34, 35, 38, 39, 41, 44, 46, 49 and 50. 'Slightly disagree' scores one point and 'strongly disagree' scores two points on the following items: 3, 11, 15, 16, 22, 27, 32, 47, 51 and 54. The maximum attainable score for this domain is 56.

5.2.2. *Study 1 Results*

5.2.2.1. EQ-C and SQ-C correlations

To examine the relationship between the EQ-C and SQ-C, a correlation was performed for all groups together, yielding a small but significant negative correlation ($r=-0.12$, $p<0.001$).

5.2.2.2. Internal consistency

Cronbach's α coefficients were calculated for the measure as a whole, as well as for each domain separately. Results showed high coefficients for the questionnaire as a whole ($\alpha=0.85$) for the sexes combined and for girls ($\alpha=0.84$) and boys ($\alpha=0.86$) separately. For EQ-C items, internal consistency was also high ($\alpha=0.93$) for the sample combined as well as in girls ($\alpha=0.91$) and boys ($\alpha=0.92$) separately. The internal consistency for the SQ-C was acceptable for the sexes combined ($\alpha=0.78$) and in girls ($\alpha=0.77$) as well boys ($\alpha=0.78$) separately.

5.2.2.3. Test-retest reliability

A random selection of 500 participants was asked to complete a second copy of the EQ-C and SQ-C six months later, to examine test-retest reliability, resulting in 258 test-retest pairs (133 girls, 125 boys). For the EQ-C, the intraclass correlation between the two tests was 0.86 (single measures) ($p<0.001$). The intraclass correlation for the SQ-C between the two tests was 0.84 (single measures) ($p<0.001$).

5.2.2.4. Sex differences

Table 5.1 shows means, standard deviations and t-test results for all cases by group. Examination of sex differences in the typical group showed that typical boys and girls differed significantly on both the EQ-C and SQ-C. Girls scored higher on the EQ-C,

and boys scored higher on the SQ-C. No significant differences in EQ-C and SQ-C scores were found between boys and girls in the ASC group. These children were therefore combined into a single group in subsequent analyses.

Table 5.1. Mean scores for EQ-C and SQ-C by Group

		EQ-C Total	SQ-C Total
Typical Group (*n*=1256)	x (SD)	37.70 (9.81)	24.11 (8.02)
Typical Girls (*n*=675)	x (SD)	40.16 (8.89)	22.64 (7.94)
Typical Boys (*n*=581)	x (SD)	34.84 (10.07)	25.81 (7.79)
ASC Group (*n*=265)	x (SD)	13.97 (6.82)	27.43 (9.20)
ASC Girls (*n*=46)	x (SD)	15.43 (6.27)	26.11 (9.11)
ASC Boys (*n*=219)	x (SD)	13.66 (6.90)	27.71 (9.22)
Typical Girls vs. Typical Boys	t	9.95**	7.12**
ASC Girls vs. ASC Boys	t	1.61	1.08
Typical Boys vs. ASC Group	t	30.69**	2.65*

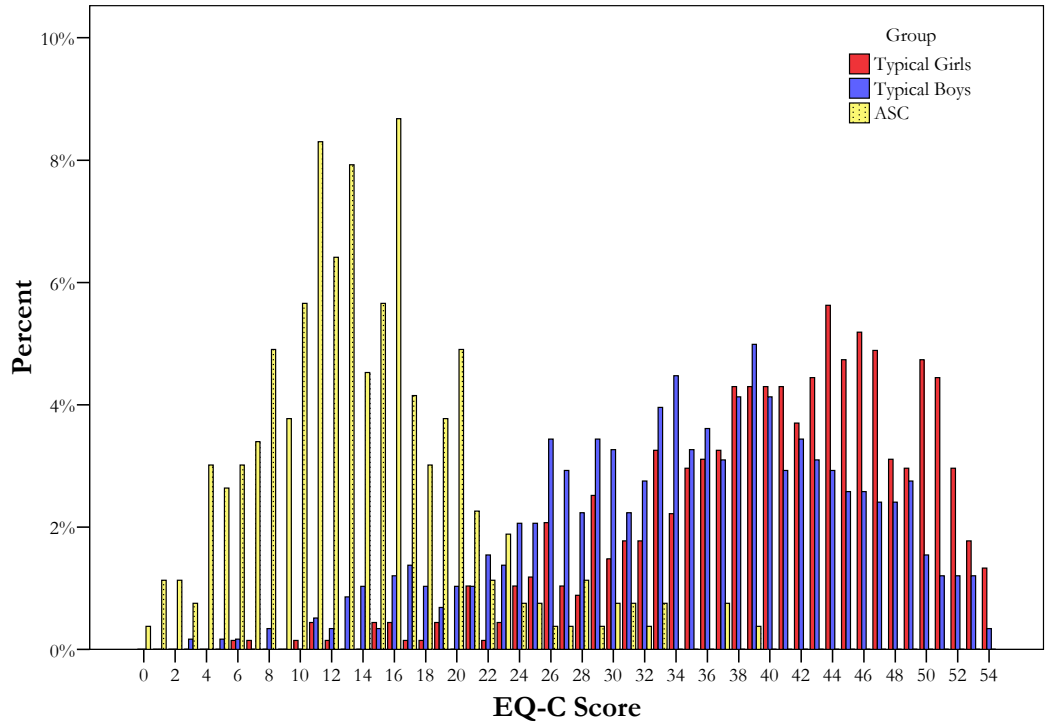
*p<0.01, ** p<0.001

5.2.2.5. The EQ-C

Analysis of EQ-C scores for all groups showed that the distribution was not skewed (skewness<1). A one-way between subjects ANOVA was conducted to examine if group (typical girls, typical boys and ASC) differences existed. There was a significant difference between groups ($F_{2,1518}$=806.89, p<0.001). Post hoc Tukey HSD tests showed significant differences between all three groups (all p<0.001) with typical girls scoring the highest (M=40.16, SD=8.89), followed by typical boys (M=34.84, SD=10.07) and the ASC group (M=13.97, SD=6.82).

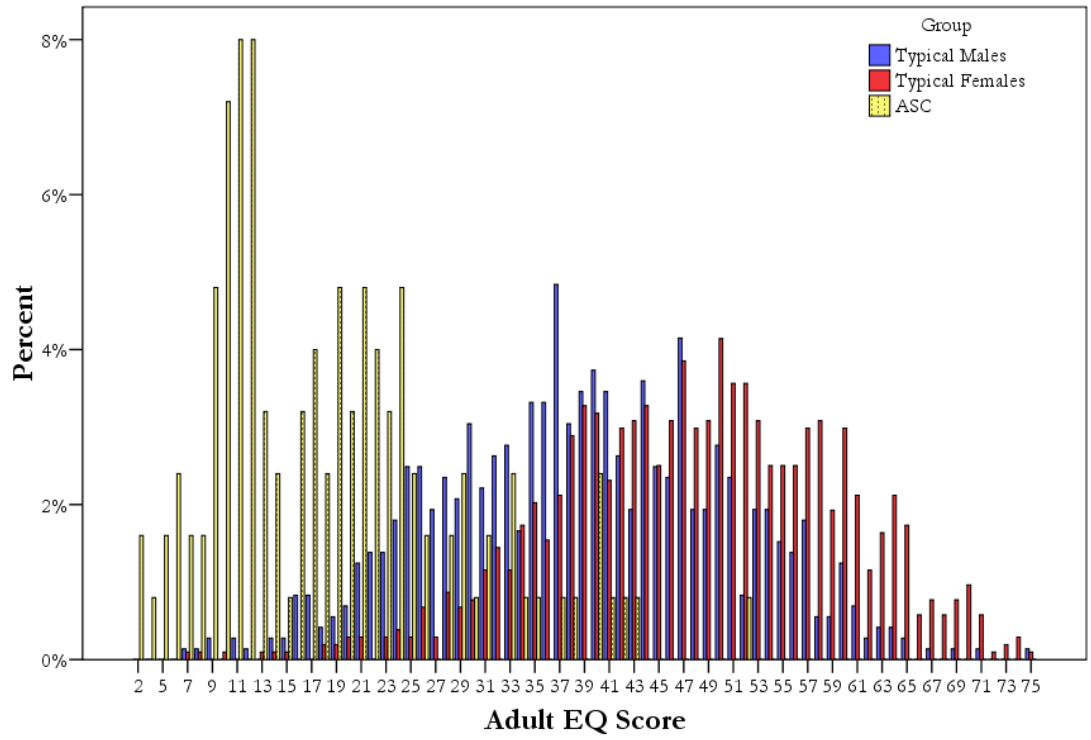
For a visual representation of the scoring patterns on the EQ-C between each group, see Figure 5.1. In order to compare the scoring patterns observed in EQ scores between children and adults, Figure 5.2 shows the scoring patterns shown on the Adult version of the EQ (Wheelwright et al., 2006).

Figure 5.1. Group scoring patterns on the EQ-C



Note: Girls with ASC scored did not score differently than boys with ASC on the EQ-C, therefore boys and girls with ASC were combined.

Figure 5.2. Group scoring patterns on the Adult EQ

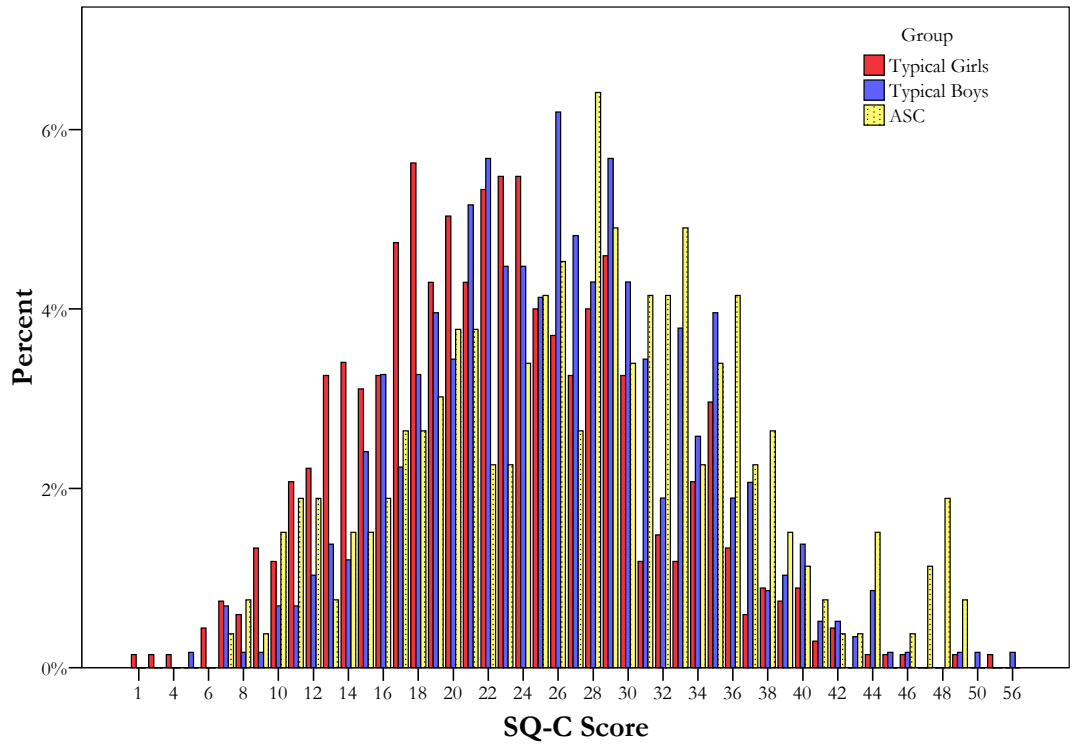


Data from: Wheelwright, S., Baron-Cohen, S., Goldenfeld, N., Delaney, J., Fine, D., Smith, R., et al. (2006). Predicting Autism Spectrum Quotient (AQ) from the Systemising Quotient-Revised (SQ-R) and Empathy Quotient (EQ). Brain Research, 1079, 47-56.

5.2.2.6. The SQ-C

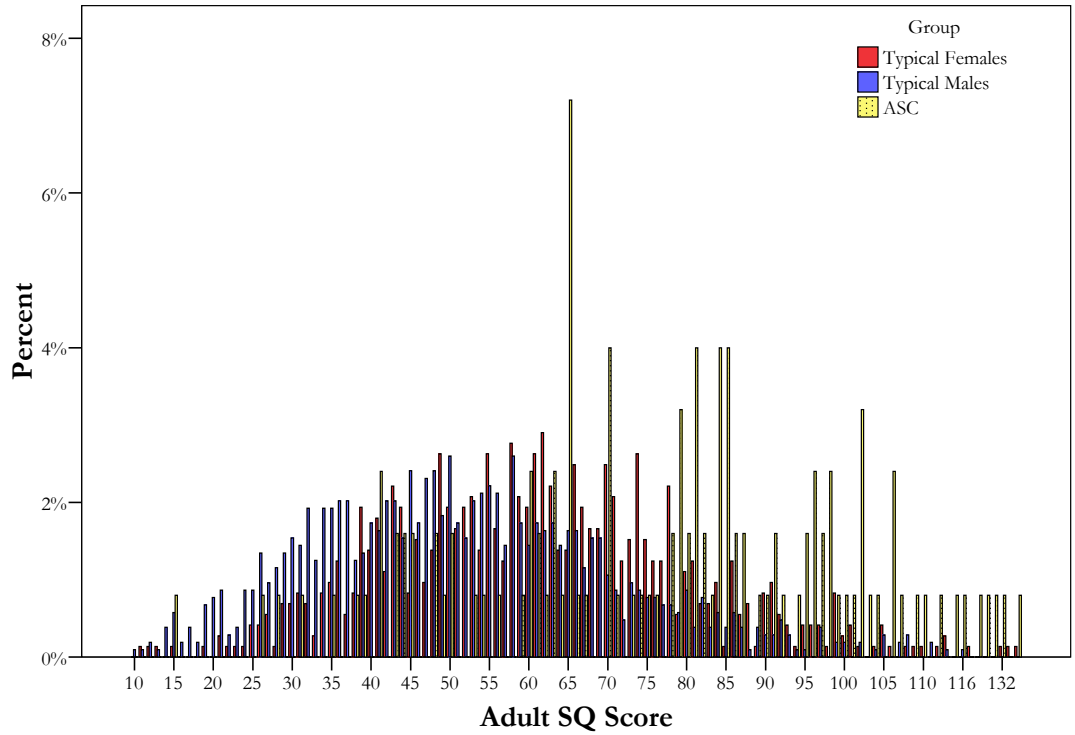
Analysis of SQ-C scores showed that the distribution was not skewed (0.27). Differences between the groups were analysed using a one-way between subjects ANOVA. The ANOVA revealed a significant main effect for ASC diagnosis ($F_{2,1518}$=42.16, p<0.001). Tukey HSD pairwise comparisons revealed significant differences between the groups (all p<0.001), with the ASC group (M=27.43, SD=9.20) scoring the highest, followed by typical boys (M=25.81, SD=7.79) who were followed by typical girls (M=22.64, SD=7.94). Figure 5.3 shows the SQ-C scoring patterns for each group. Figure 5.4 shows Adult SQ scoring patterns.

Figure 5.3. Group scoring patterns on the SQ-C



Note: Girls with ASC did not score differently than boys with ASC on the SQ-C, therefore boys and girls with ASC were combined.

Figure 5.4. Group scoring patterns on the Adult SQ



Data from: Wheelwright, S., Baron-Cohen, S., Goldenfeld, N., Delaney, J., Fine, D., Smith, R., et al. (2006). Predicting Autism Spectrum Quotient (AQ) from the Systemising Quotient-Revised (SQ-R) and Empathy Quotient (EQ). Brain Research, 1079, 47-56.

5.2.2.7. Brain Types

In order to examine the proportion of children scoring in each of 5 defined cognitive 'brain types' predicted by the E-S theory (Type B, Type E, Type S, Extreme S and Extreme E), scores were transformed using a method reported elsewhere (Goldenfeld et al., 2005; Wheelwright et al., 2006). First, standardised scores were calculated for both EQ-C and SQ-C for the entire sample using the following formulae:

$$E_{Standardised}=(EQ\text{-}C_{Observed} - EQ\text{-}C_{GroupMean})/EQ\text{-}C_{MaxScore}$$

$$S_{Standardised}=(SQ\text{-}C_{Observed} - SQ\text{-}C_{GroupMean})/SQ\text{-}C_{MaxScore}$$

The typically developing group means were used for the EQ-C (M=37.70; SD=9.81) and SQ-C (M=24.11; SD=8.02). The standardised E and S variables were used to produce a difference score (D). This new variable was defined as follows:

$$D_{(S-E)} = (S - E)/2$$

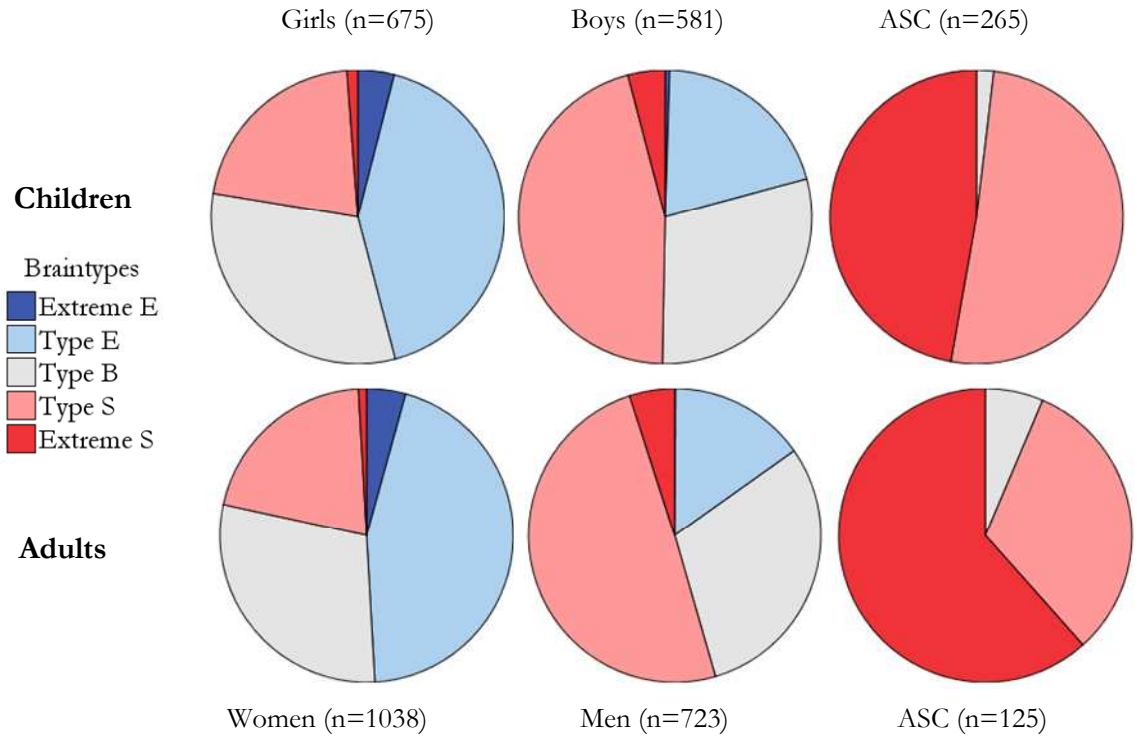
Brain types were numerically assigned according to the percentiles of the typically developing group on the D axis. The lowest scoring 2.5% on the D axis were classified as Extreme Type E. Participants who scored between the 2.5th and 35th percentiles were classified as Type E. Those scoring between the 35th and 65th percentile were classified as Type B. Type S was defined by scores between the 65th and 97.5th percentile, and the top 2.5% were classified as Extreme Type S. See Table 5.2 for the proportion of participants from the sample with each brain type. Table 5.2 also shows data for adult females, adult males and adults with ASC. Figure 5.5 shows a visual representation of the brain type proportions for children and adults.

Table 5.2. Percent of children and adults with each brain type measured in D

Brain Type	D Percentile (per)	Brain Type Boundary	Group					
			Girls n=675	Boys n=581	ASC Children n=265	Women* n=1038	Men* n=723	ASC* n=125
Extreme E	per < 2.5	D <-0.205	4.0	0.5	0	4.3	0.1	0
Type E	2.5 ≤ per <35	-0.205≤ D <-0.050	41.9	20.3	0	44.8	15.1	0
Type B	35 ≤ per <65	-0.050≤ D <0.037	31.7	29.5	1.9	29.3	30.3	6.4
Type S	65 ≤ per <97.5	0.037≤ D <0.260	21.2	45.6	50.9	20.7	49.5	32.0
Extreme S	per ≥ 97.5	D ≥0.260	1.2	4.1	47.2	0.9	5.0	61.6

*Data from Wheelwright, Baron-Cohen, Goldenfeld, Delaney, Fine, Smith, Weil, Wakabayashi, 2006

Figure 5.5. Brain type proportions of children and adults



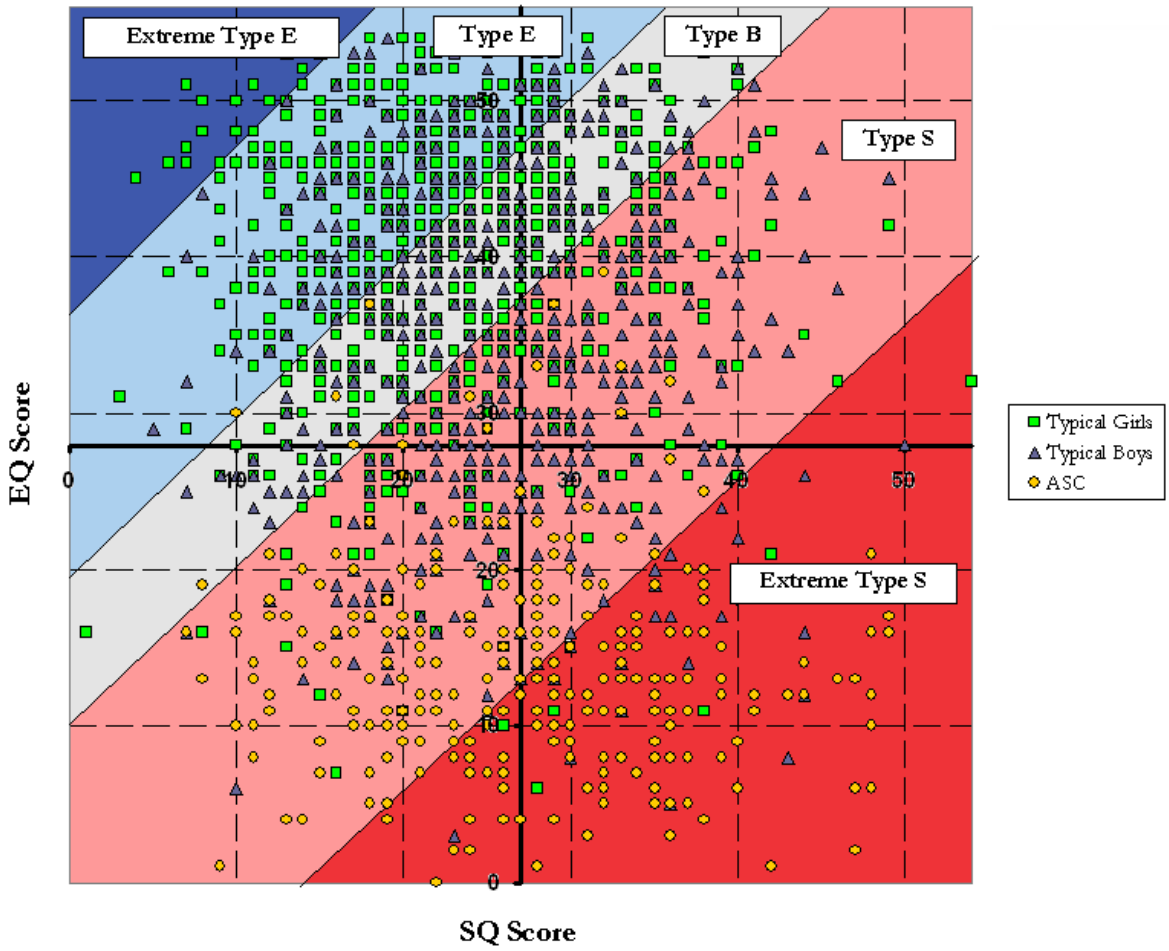
*Adult data from Wheelwright, Baron-Cohen, Goldenfeld, Delaney, Fine, Smith, Weil, Wakabayashi, 2006

A one-way between subjects ANOVA was used to test for group differences in D scores between girls, boys and children with ASC. Results showed a significant effect of group ($F_{2,1518}$=642.01, $p<0.001$). Tukey HSD post hoc tests show that all groups differed significantly (all $p<0.001$) from each other with typical girls (M=-0.36, SD=0.11) tending to lie on the lower end of the brain type spectrum (Extreme E or Type E), followed by typical boys (M=0.04, SD=0.12), and children with ASC (M=0.25, SD=0.11) showing a tendency to fall on the higher end (Type S or Extreme S) of the spectrum.

See Figure 5.5 for a visual representation of the brain-types. Note that the boundaries were based on percentiles calculated from the typically developing sample. Starting in the top left hand corner and passing along this axis, it can be seen that the highest

concentration of participants changes from typical girls to typical boys and children with ASC.

Figure 5.6. Results translated back into raw scores on the EQ-C and SQ-C



5.3. Study 2: Foetal testosterone and the EQ-SQ-C

5.3.1. *Study 2 Methods*

5.3.1.1. Study 2 Participants

The combined EQ-C and SQ-C was sent to mothers of typically developing children who are participating in the Cambridge Foetal Testosterone Project (n=456 mothers contacted), and was collected in collaboration with Emma Ashwin, graduate researcher.

The EQ-SQ-C was completed by n=212 mothers. Questionnaires with more than five blank items were considered incomplete and these data were discarded in subsequent analyses (n=4). If five or fewer answers were missing, the score was corrected for missing items (n=3), resulting in a total of n=208 (113 boys, 95 girls) children with complete data.

5.3.1.2. Predictor variables

Chapter 2 provides a detailed description of the predictor variables utilised in this study. FT level was the predictor of greatest interest in this study. The control variables that were included in the subsequent analyses were gestational age at amniocentesis, maternal age, level of education obtained by the parents, presence of older siblings and child's age.

5.3.2. *Study 2 Results*

Examination of the univariate distributions revealed that foetal testosterone level was positively skewed, and was the only predictor variable with a distribution that deviated significantly from the Gaussian distribution. Two female outliers in FT levels (individuals who scored three or more standard deviations from the mean) were observed. These outlying values were replaced using a windsorizing procedure, where the extreme values are replaced by the highest observed level within three standard deviations from the mean (0.80 nmol/L). No outliers were found when boys' FT levels were examined. Windsorized FT levels showed no outliers and acceptable skewness statistics for both boys and girls, and are used in subsequent analyses.

Table 5.3 presents the means and standard deviations for each sex separately, as well as combined for predictor variables, EQ-C, SQ-C and D scores.

Table 5.4 shows the correlation coefficients for predictor and outcome variables. Tables 5.5 and 5.6 show correlation coefficients for girls and boys separately.

Table 5.3. Descriptive statistics for Study 2

	Combined Group				Girls				Boys				
Variable	n	M	SD	Range	n	M	SD	Range	n	M	SD	range	Cohen's d
^FT level (nmol/L)**	208	0.60	0.44	0.05-2.05	95	0.32	0.27	0.05-1.75	113	0.83	0.41	0.10-2.05	1.47
Gestational Age	140	16.32	1.97	13-22	64	16.44	2.52	13-22	76	16.22	1.36	13-20	0.12
Child Age	208	7.19	1.03	5.17-9.92	95	7.08	1.04	5.25-8.83	113	7.29	1.01	5.17-9.92	0.20
Maternal Age	178	41.20	4.52	29.42-53.23	81	41.22	4.60	29.42-53,15	97	41.18	4.50	31.67-53.23	0.01
Parental Education	177	3.24	1.00	1-5	81	3.05	0.82	1-5	96	3.41	1.11	1-5	0.37
EQ-C**	208	34.81	9.95	7-54	95	37.65	8.14	17-54	113	32.42	10.72	7-52	0.55
SQ-C**	208	25.36	8.18	6-47	95	23.40	8.36	6-47	113	27.00	7.69	10-44	0.49
D**	208	0.04	0.12	-0.18-0.41	95	-0.01	0.11	-0.18-0.37	113	0.07	0.11	-0.16-0.41	0.73

^Indicates raw values

* Sex difference significant at the $p<0.05$ level

** Sex difference significant at the $p<0.01$ level

Table 5.4. Correlation matrix for all cases

	FT Level	Sex	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	EQ-C Total	SQ-C Total
Sex	.64**	--	--	--	--	--	--	--	--	--
Gestational Age	-.08	-.05	--	--	--	--	--	--	--	--
Child Age	.04	.10	.11	--	--	--	--	--	--	--
Maternal Age	-.03	.01	-.31**	.09	--	--	--	--	--	--
Parent Education	.10	.18*	-.15	-.08	.16*	--	--	--	--	--
Older Sister	-.05	-.03	-.01	-.09	.04	-.06	--	--	--	--
Older Brother	-.06	-.08	.04	-.10	-.04	-.09	.24**	--	--	--
EQ-C	-.21**	-.26**	.05	-.02	.09	-.02	.27	-.14	--	--
SQ-C	.31**	.22**	-.01	.14*	.14	-.11	.11	.32*	.04	--
D	.36**	.35**	-.04	.11	.01	-.06	-.11	.04	-.77**	.60**

* $p<0.05$, ** $p<0.01$

Table 5.5. Correlation matrix for Girls

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	EQ-C Total	SQ-C Total
Gestational Age	.00	--	--	--	--	--	--	--	--
Child Age	.06	.19	--	--	--	--	--	--	--
Maternal Age	.00	-.34**	.01	--	--	--	--	--	--
Parent Education	.08	-.18	-.01	.13	--	--	--	--	--
Older Sister	.01	.14	-.03	-.11	-.12	--	--	--	--
Older Brother	.09	.16	-.16	-.06	-.10	.31**	--	--	--
EQ-C	-.18	.12	.04	-.09	-.05	.03	-.09	--	--
SQ-C	.50**	.01	.14	.13	-.27	.08	.14	-.02	--
D	.47**	-.08	.07	.15	-.16	.04	.16	-.72**	.71**

* $p<0.05$, ** $p<0.01$

Table 5.6. Correlation matrix for Boys

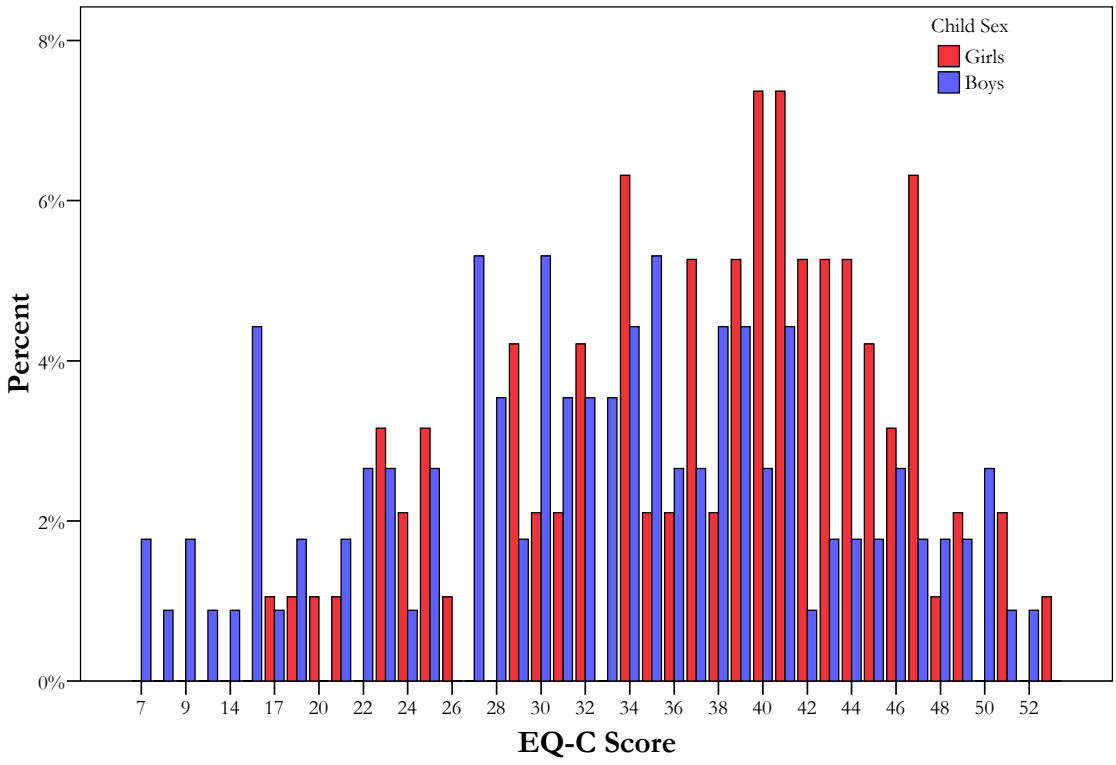
	FT Level	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	EQ-C Total	SQ-C Total
Gestational Age	-.13	--	--	--	--	--	--	--	--
Child Age	-.08	.02	--	--	--	--	--	--	--
Maternal Age	-.04	-.28*	.15	--	--	--	--	--	--
Parent Education	-.06	-.10	-.14	.18	--	--	--	--	--
Older Sister	-.06	-.20	-.13	.17	-.03	--	--	--	--
Older Brother	-.06	-.17	-.01	-.02	-.07	.16	--	--	--
EQ-C	-.01	-.06	-.01	.21*	.07	.16	.02	--	--
SQ-C	.13	-.01	.11	.15	-.08	-.14	.01	.19*	--
D	.09	.04	.08	-.10	-.11	-.23	-.02	-.79**	.45**

* $p<0.05$, ** $p<0.01$

5.3.2.1. EQ-C Scores

Examination of univariate distributions indicated that the distribution for EQ-C scores was not skewed, (skewness<1). See Figure 5.7 for the distribution of EQ-C scores.

Figure 5.7. Distribution of EQ-C scores



No significant sex differences were found for any of the predictor variables except FT level. Scores on the EQ-C showed significant sex-differences, $t(204.01)=3.91$, $p<0.001$, equal variances not assumed, with girls ($M=37.65$, $SD=8.14$) scoring higher than boys ($M=32.42$, $SD=10.72$).

5.3.2.2. Internal Consistency

Cronbach's α coefficients for EQ-C score in this sample was high for the boys ($\alpha=0.89$) and girls ($\alpha=0.82$) separately and combined ($\alpha=0.88$). Split half reliability for the

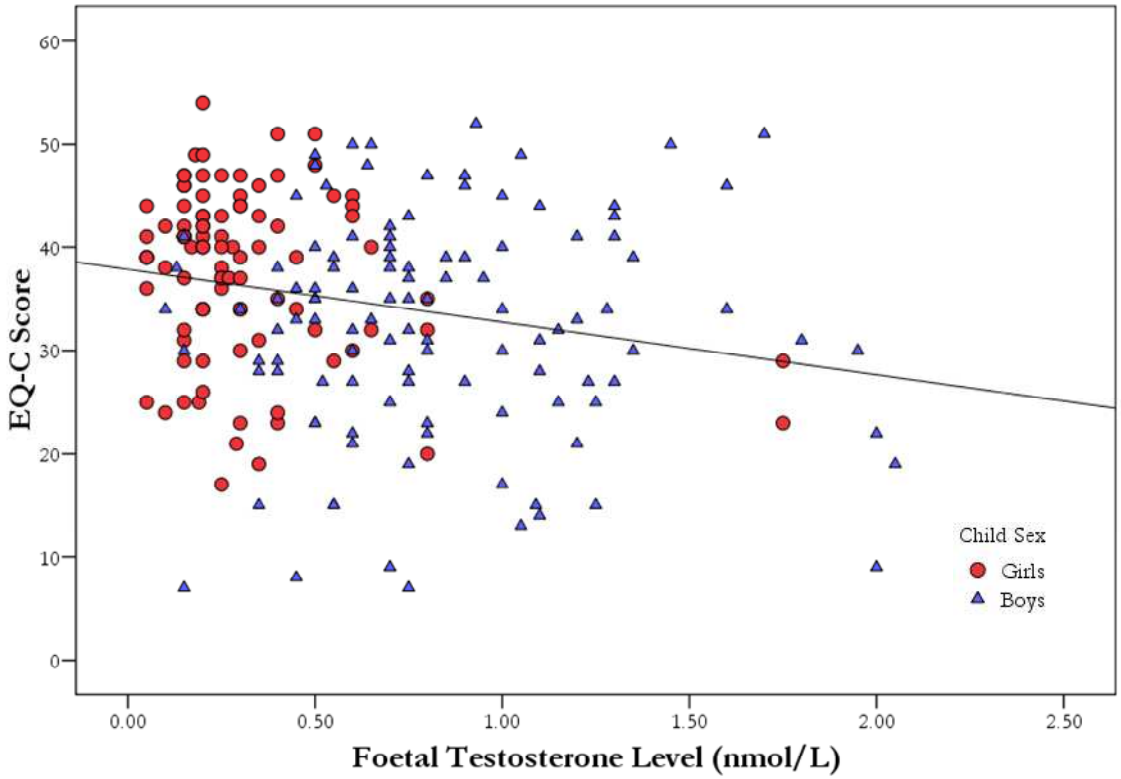
measure as a whole was acceptable for boys and girls together (0.75), and in boys (0.77) and girls (0.64) alone.

Table 5.7. Final regression model for EQ-C scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
EQ-C	Older sister	0.01	0.01	3.55	2.23	0.11	p>0.05
	Older brother						p>0.05
	Sex	0.08	0.07	2.63	0.67	0.26	P<0.001
		Girls only					
EQ-C	No significant predictors						
		Boys only					
EQ-C	No significant predictors						

For the regression analysis, the predictor variables that met the entry criteria were Sex ($r=-0.26$, $p<0.001$) and FT ($r=-0.20$, $p<.01$). No suppressor variables were found. The regression analysis excluded FT level as a predictor variable. Within sex analyses were also conducted for EQ-C scores to further investigate the relationship between EQ-C score and FT for boys and girls separately. No significant correlations were found between EQ-C and FT level for boys or girls. Figure 5.8 shows the relationship between FT level and EQ-C scores.

Figure 5.8. FT level and EQ-C score

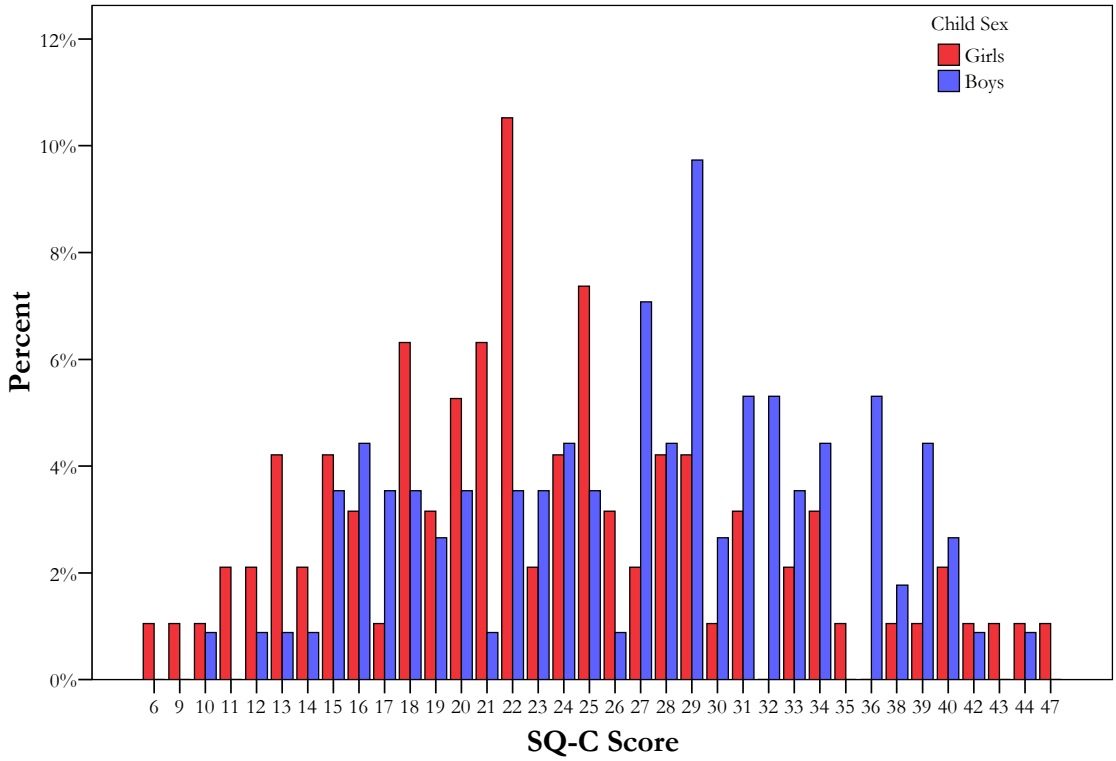


Note: A significant correlation was observed between FT level and EQ-C scores; however, FT was not retained in the regression analysis as a significant predictor.

5.3.2.3. SQ-C Scores

Examination of univariate distributions indicated that the distribution for SQ-C scores was not skewed, (skewness<1). See Figure 5.9 for the distribution of SQ-C scores. SQ-C scores showed significant sex-differences, $t(206)=3.23$, $p<0.01$ (equal variances assumed), with boys ($M=27.00$, $SD=7.69$) scoring higher than girls ($M=23.40$, $SD=8.36$).

Figure 5.9. Distribution of SQ-C Score



5.3.2.4. Internal Consistency

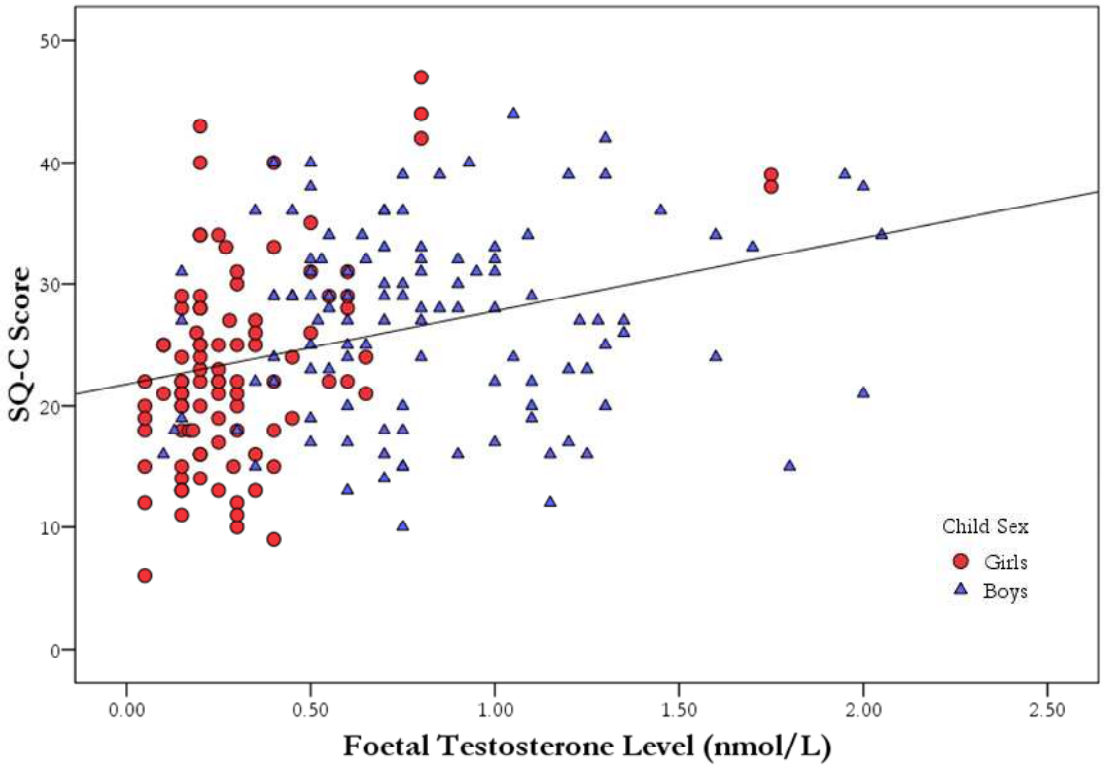
Cronbach's α was calculated for the SQ-C and demonstrated acceptable internal consistency for the sexes together (α=0.77) and for girls (α=0.76) and boys (α=0.77) separately. Split half reliability was good for the entire sample (0.76) and for girls (0.77) and boys (0.77).

Table 5.8. Final regression model for SQ-C scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
SQ-C	Child age	0.03	0.03	1.12	0.57	0.14	$p<0.05$
	Parent education			1.09	0.59	0.13	$p>0.05$
	FT level	0.13	0.10	6.66	1.49	0.32	$p<0.001$
		Girls only					
SQ-C	Parent education	0.09	0.09	3.15	0.99	0.31	$p<0.01$
	Older sisters			0.01	2.78	0.04	$p>0.05$
	Older brothers			0.98	2.78	0.04	$p>0.05$
	FT level	0.32	0.23	22.34	4.36	0.49	$p<0.001$
		Boys only					
SQ-C	No significant predictors						

Table 5.8 shows the final regression results for SQ-C scores. The predictor variables that correlated with SQ-C scores at $p<0.20$ were gestational age ($r=0.22$, $p<0.20$) and parent education level ($r=-0.11$, $p<0.20$). These variables were included in the first stage using the enter method. The final model retained FT level (F-change=8.88, $p<.001$, $\Delta R^2=0.10$). See Figure 5.10 for a visual representation of the relationship between FT level and SQ-C scores.

Figure 5.10. FT level and SQ-C score



Within sex analyses were also conducted. For girls, parent education level (r=-0.27, p<0.20), presence of older brothers (r=0.14, p<0.20) and sisters (suppressor) were included in the hierarchical regression analysis using the enter method in the first block. The final regression model retained FT level (F-change=8.95, p<0.001, ΔR^2=0.23). No significant correlations were found between SQ-C and the predictor variables in boys. Therefore, regression analyses were not conducted.

5.3.2.5. Brain Types

Brain types were calculated by using a method described elsewhere (Goldenfeld et al., 2005; Wheelwright et al., 2006). The typically developing group mean scores used were (M=37.70; SD=9.81) for the EQ-C and (M=24.11; SD=8.02) for the SQ-C. Standardised E and S variables were used to produce a difference score (D) according to the equations in section 5.2.2.7. Significant sex differences were observed in D scores

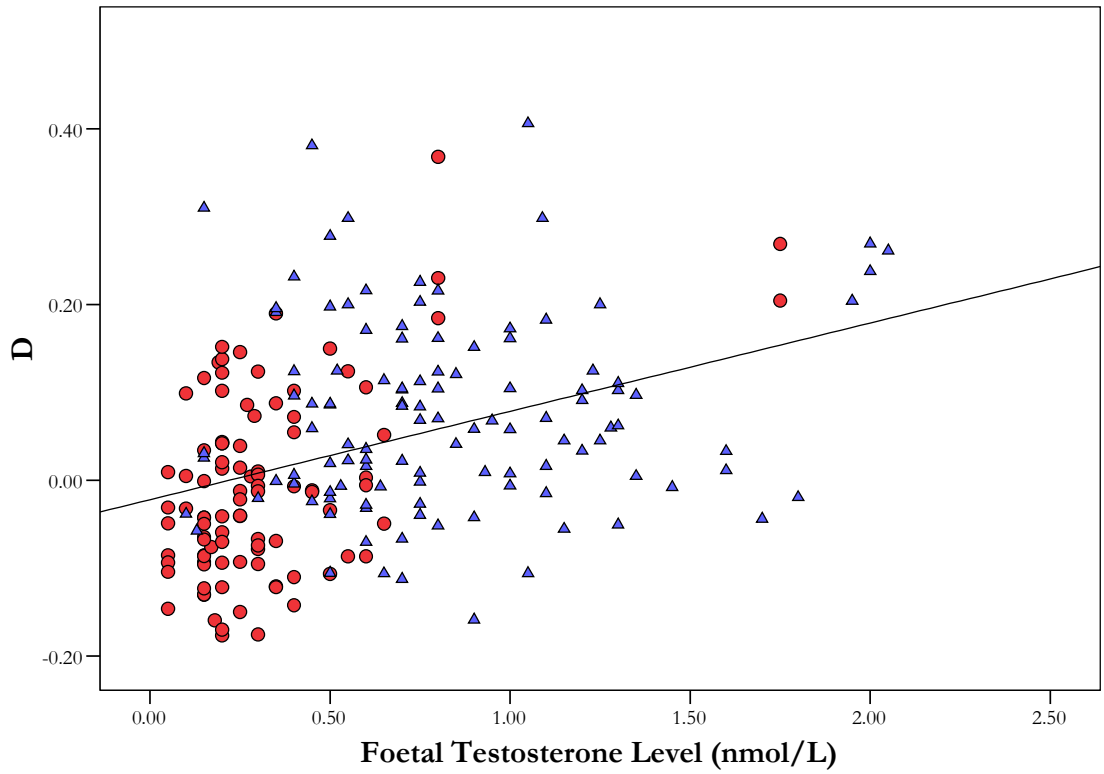
with boys (M=0.07, SD=0.11) showing more masculinised brain types than girls (M=-0.01, SD= 0.11), t(206)=5.35, p<0.001 (equal variances assumed).

Table 5.9. Final regression model for D scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
D	Child age	0.03	0.03	0.01	0.01	0.06	p>0.05
	Older sisters			0.04	0.02	0.11	p>0.05
	Older brothers			0.04	0.03	0.08	p>0.05
	FT level	0.15	0.12	0.14	0.03	0.53	p<0.001
	Sex	0.18	0.03	0.07	0.02	0.59	p<0.001
	FT level X Sex	0.23	0.05	0.12	0.03	0.67	p<0.001
		Girls only					
D	Maternal age	0.08	0.08	0.04	0.02	0.18	p<0.01
	Parent education			0.03	0.01	0.20	
	Older sisters			0.01	0.03	0.20	p>0.05
	Older brothers			0.03	0.04	0.08	p>0.05
	FT level	0.30	0.22	.027	0.06	0.48	p<0.001
		Boys only					
D	No significant predictors						

Table 5.9 shows the final regression results for D scores. Child age (r=0.11, p<0.20), presence of older sisters (r=-0.11, p<0.20) and brothers (suppressor) met criteria for entry into the hierarchical regression analysis in the first stage. A significant model emerged retaining FT level, Sex and the FT/Sex interaction (F-change=10.18, p<0.001, R^2=0.23) (see Figure 5.11).

Figure 5.11. FT level and D



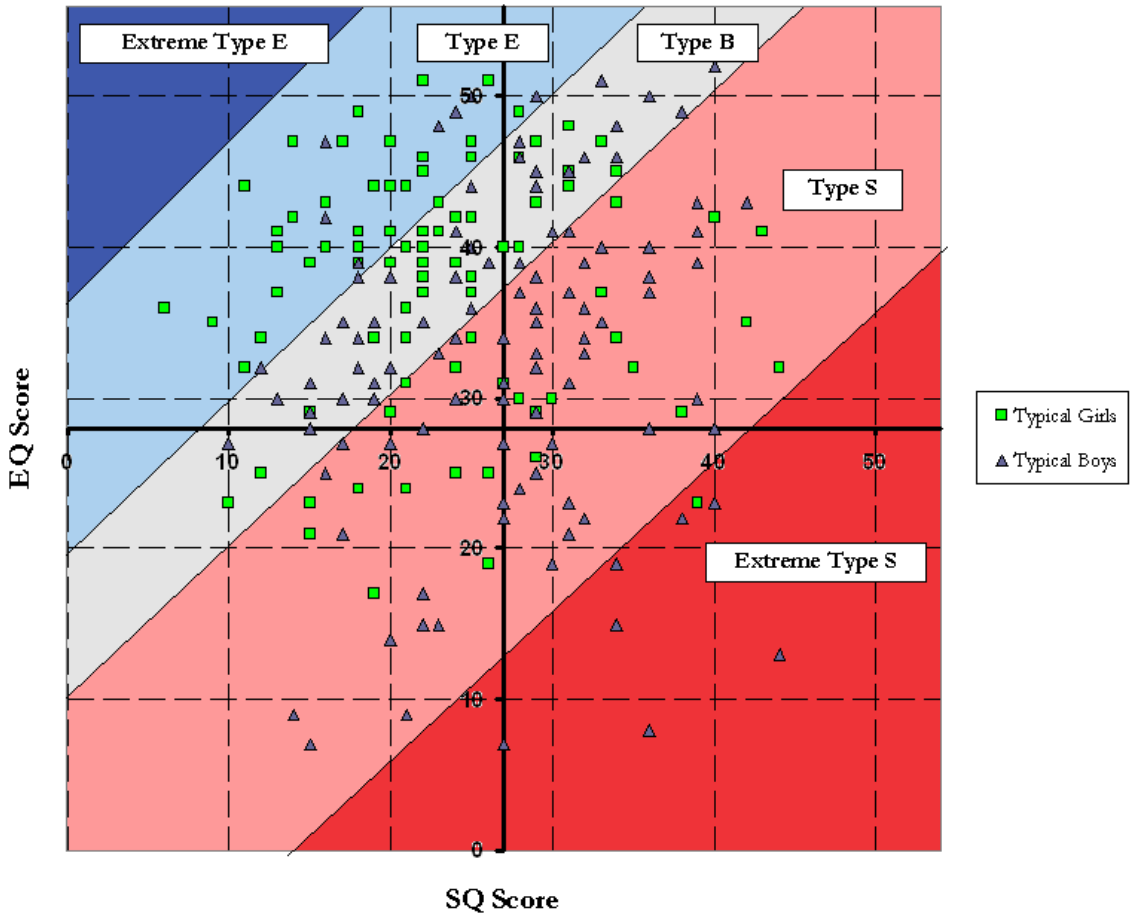
Within sex analyses for girls showed that maternal age ($r=0.47$, $p<0.001$), parent education level ($r=0.47$, $p<0.001$), older sisters ($r=0.47$, $p<0.001$) and older brothers (suppressor) met criteria for inclusion in the first stage of the regression analysis. The regression analysis included FT level in the final model (F-change=6.37, $p<0.001$, $\Delta R^2=0.22$). No significant correlations were found between D and the predictor variables for boys, and regression analyses were not conducted.

Brain types for each of the participants in Study 2 were calculated using the brain type boundaries obtained from the sample of n=1256 typically developing children in Study 1. See Table 5.10 for the percent of participants from this sample with each brain type and Figure 5.12 for a visual representation of the brain types observed in Study 2.

Table 5.10. Percent of children with each brain type

Brain Type	D Percentile (per)	Brain Type Boundary	Group	
			Girls n=95	Boys n=113
Extreme E	per < 2.5	D <-0.205	0	0
Type E	2.5 ≤ per <35	-0.205≤ D <-0.050	37.9	8.8
Type B	35 ≤ per <65	-0.050≤ D <0.037	31.6	33.6
Type S	65 ≤ per <97.5	0.037≤ D <0.260	28.4	49.6
Extreme S	per ≥ 97.5	D ≥0.260	2.1	7.1

Figure 5.12. Braintypes



Note: Brain type boundaries from Study 1

5.4. Discussion

Study 1 reports the development of parent-report versions of the Empathising (EQ-C) and Systemising Quotients (SQ-C). These were administered to n=1256 typically developing children and were found to have high internal consistency. The questionnaires also demonstrated good test-retest reliability at an interval of six months.

Sex differences were found in both empathising and systemising. Girls on average scored higher than boys on the EQ-C, and boys on average scored higher than girls on the SQ-C. Results including a group of children with a diagnosis of ASC (n=265) confirm the scoring patterns observed in adults can also be found in children. As predicted, children with ASC scored significantly lower on the EQ-C than controls and significantly higher on the SQ-C than typical boys. The scores for the ASC group on the SQ-C are also consistent with the 'normal or superior' level of systemising suggested by the extreme male brain (EMB) theory. The results indicate a difference in brain type, rather than an overall cognitive disadvantage. It is also interesting to note that no significant sex differences were found in the ASC population for empathising and systemising, unlike typically developing boys and girls. These results need to be further explored using a larger sample of girls with ASC. Smaller sample sizes are often an inherent difficulty in studying girls with ASC, due to the much smaller proportion of girls diagnosed with these conditions.

Results also indicated that the SQ-C and EQ-C were weakly but significantly inversely correlated. The negative correlation is of a similar magnitude and direction as reported for the adult EQ and SQ (Wheelwright et al., 2006). The consistency between adults and children was also seen in the proportion of individuals falling in each brain type (as shown in Table 5.2). These results suggest that differences in empathising and systemising are present early in childhood and are consistent with those found in adulthood.

Study 2 examined the relationship between FT levels, brain type, EQ-C and SQ-C scores. Results for girls and boys together showed that FT levels were significantly negatively correlated with EQ-C scores. However, the subsequent regression analysis showed a main effect of Sex, but not FT levels. The strong correlation between Sex and FT means that FT cannot be ruled out as a factor in producing the observed sex difference, but it is clear that the effect of Sex is larger than that which would be predicted by FT alone. The correlation observed between FT and EQ-C may in part be due to a larger variation in FT levels for boys compared to girls in this sample. It has been suggested that genetic factors may influence EQ score in adults (Chakrabarti et al., submitted; Skuse, 2000), and these might also be related to sex hormones. It is hoped that these relationships might be investigated in future studies.

A positive association between FT levels and SQ-C scores was also found when boys and girls were examined together. Sex was not included in the final regression model for SQ-C score, suggesting that FT levels play a greater role than the child's sex in systemising ability. When sexes were examined separately, a significant relationship between FT and SQ-C scores was found in girls, but the correlation found when boys were examined alone was not significant. It is possible that girls are more sensitive to changes in FT levels or that the correlation in boys might be significant in a larger sample. Perhaps future research could further examine these within sex relationships in a larger sample of children using more objective measures.

Researchers have also stressed the importance of context when examining sex differences (Hyde, 2005), and a questionnaire-based study makes the measurement of such variables difficult. Against the drawbacks of parental report, an advantage is that mothers have the opportunity to judge their children's traits, skills, strengths and weaknesses in a variety of contexts over an extended period of time. It will be important to validate the questionnaire scores against performance measures in a more controlled setting.

In Study 1, typically developing boys scored significantly higher than typical girls on the SQ-C and significantly lower on the EQ-C. Children with ASC scored significantly higher on the SQ-C, and significantly lower on the EQ-C compared to typical boys, providing further support for the notion that individuals with ASC show a 'hyper-masculinised' cognitive profile. This study also showed that children exhibit very similar patterns of empathising and systemising to those found in adults. Pre-pubertal children show relatively low levels of hormones (Collaer & Hines, 1995). If hormones do play a role in empathising and systemising, these indirect results suggest that prenatal or neonatal factors may be involved in these patterns.

In addition, the examination of the relationship between FT levels and these new measures in Study 2 revealed that FT levels showed a significant correlation to both EQ-C and SQ-C scores. Hierarchical regression analysis revealed that FT was the only significant predictor of systemising when the sexes were examined together. A combined sex analysis showed a significant negative correlation between FT level and performance on the EQ-C, but FT level was not retained in the final regression model.

The current results lend further support to the E–S theory of sex differences showing that sexual dimorphism is present in children and these remain consistent throughout adulthood. Children with ASC showed a 'hyper-masculinised' profile, supporting the EMB theory. The current findings also suggest that systemising may be related to prenatal exposure to FT.

Chapter 6: Foetal testosterone and autistic traits

Experimental evidence has suggested a link between prenatal exposure to testosterone and masculinisation of certain behaviours. Other evidence supports the characterisation of ASC as an extreme manifestation of certain male typical behaviours. The aim of this study is to examine the relationship between prenatal exposure to testosterone and the development of autistic traits in children. Evaluation of autistic traits was measured using the Childhood Autism Spectrum Test (CAST) and a modified version of the Autism Spectrum Quotient for children (AQ-Child). Although the CAST was specifically developed to screen for ASC in children, it may be less useful for measuring the range of autistic traits in research studies because scores are skewed. The AQ has been developed as a measure of autistic traits in a wider population of adults and adolescents and is more useful for research because it is close to normally distributed. In the first part of this chapter, we report how the AQ was modified for children, and Study 1 reports the development of this measure of autistic traits in children. The AQ-Child was administered to children with an ASC (n=540) and a general population sample (n=1225). Results showed significant differences in scores between children with an ASC diagnosis and typically developing children. In Study 2, the link between FT levels and CAST and AQ-Child score was examined in n=235 children. Sex differences were found for both measures, with boys scoring higher than girls. FT levels were positively associated with higher scores on the CAST and AQ-Child. These results provide support for the EMB theory of autism, and for a role for foetal androgens in the development of autistic traits.

6.1. Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (1994), a diagnosis of autism is determined on the basis of deficits in three criterion domains: 1) reciprocal social behaviour; 2) repetitive behaviours (or a restricted range of interests) and 3) language development. Other common pervasive developmental disorders include Pervasive Developmental Disorder Not Otherwise Specified (PDD/NOS) and Asperger syndrome (AS), which are characterised by milder deficits in reciprocal social behaviour and can occur with or without obvious impairment in the other two criterion domains for autism.

Recent research has suggested that autism represents the upper extreme of a collection of traits that may be continuously distributed (Baron-Cohen et al., 2001a; Constantino et al., 2004; Constantino et al., 2000; Constantino & Todd, 2003), with AS 'bridging' autism and typical development (Baron-Cohen, 1995; Baron-Cohen et al., 2001a; Frith, 1991; Wing, 1988). The continuum view provides a shift away from the categorical diagnostic approach towards a quantitative approach for measuring autistic traits. Consequently, autism, High-Functioning autism (HFA), AS, and PDD/NOS are collectively referred to as Autism Spectrum Conditions (ASC).

Classic autism is generally identified by the age of 3 years (Howlin & Moore, 1997), and can be recognised in children as young as 18 months of age (Allison et al., 2008; Baird et al., 2000; Baron-Cohen et al., 1996). However, other conditions on the autism spectrum are not as easily identified, and many individuals are not recognised with conditions such as AS until much later in life (Baron-Cohen et al., 2005b; Gillberg, Nordin & Ehlers, 1996).

The Childhood Autism Spectrum Test (CAST) (Scott et al., 2002b; Williams et al., 2005) (formerly known as the Childhood Asperger Syndrome Test, renamed because it can be used for all subgroups on the autistic spectrum (Baron-Cohen et al., submitted)) is a

self-administered parental-report questionnaire specifically developed to detect more subtle manifestations of ASC, such as Asperger syndrome in primary school children. The CAST measures difficulties and preferences in social and communication skills. The questionnaire items cover topics such as the presence of rigid or repetitive behaviours, the initiation and maintenance of conversation, social interaction, play activities and interests. Studies have reported good sensitivity and specificity for ASC, moderate positive predictive value and moderate to good test-retest reliability (Allison et al., 2007; Scott et al., 2002b; Williams et al., 2005). The CAST is an established measure of autistic traits that has been validated on a large population in the United Kingdom and has been shown to be heritable (Ronald et al., 2006; Williams et al., 2005).

The Autism Spectrum Quotient (AQ-Adult) was developed to quantitatively measure traits associated with the autistic spectrum in adults of normal intelligence (Baron-Cohen et al., 2001a). The AQ was designed to be a brief, self-administered scale used to identify the degree to which an adult of normal intelligence may exhibit 'autistic traits', or what has been called 'the broader autism phenotype' (Baron-Cohen et al., 2001a; Piven et al., 1997). Results from studies using the AQ-Adult demonstrate that individuals with an ASC diagnosis score significantly higher than a sample from the general population. The AQ-Adult shows strong heritability as demonstrated in a general population twin study (Hoekstra et al., 2007), and in family genetic studies of parents of children with ASC, who score higher than unrelated individuals (Bishop et al., 2004; Wheelwright & Baron-Cohen, submitted). The AQ also predicts clinical diagnosis in adults and shows strong cross-cultural consistency (Hoekstra et al., 2008; Wakabayashi et al., 2006; Woodbury-Smith et al., 2005) and substantial heritability in the general population (Hoekstra et al., 2007). Studies of the AQ have consistently shown that scores are more normally distributed than measures such as the CAST (Baron-Cohen et al., 2001a; Hoekstra et al., 2008), suggesting it as a good measure of typical variation of autistic traits in the general population.

An adolescent version of the AQ has also been developed (AQ-Adol) (Baron-Cohen et al., 2006). Results showed that adolescents score very similarly to adults, with individuals with ASC scoring significantly higher than matched controls (Baron-Cohen et al., 2006). There is currently no version of the AQ that is appropriate for children under the age of 12 years, and the development of a children's version of the AQ is undertaken in Study 1.

The strong bias of ASC towards males has been well established (Bryson & Smith, 1998; Fombonne, 2005; Tidmarsh & Volkmar, 2003), and the extreme male brain (EMB) theory of autism proposes that ASC are an exaggeration of specific (but not all) male-typical traits (Baron-Cohen, 1999; Baron-Cohen & Hammer, 1997). Recently, this theory has been extended to explain both cognition and neuroanatomy in individuals with autism (Baron-Cohen et al., 2005a). Although the EMB theory was originally defined purely in psychometric terms, it has since been suggested that FT levels may be involved in the development of ASC and may be responsible for the biased sex ratio found in these conditions (Baron-Cohen et al., 2004).

6.1.1. *Hormones and the EMB theory*

In Chapters 2 to 5 the possibility of a link between FT levels (measured in amniotic fluid) and certain sexually dimorphic behaviours was examined. Results suggest that prenatal exposure to elevated FT levels is related to masculinisation of certain behaviours. These findings are also consistent with a series of studies that have investigated the effects of elevated foetal androgens and gender-typical behaviour (Grimshaw et al., 1995b; Knickmeyer et al., 2005a; Knickmeyer et al., 2006b; Lutchmaya et al., 2002a; Lutchmaya et al., 2002b).

In addition to measures of behaviour, elevated FT levels have also been linked to sexually dimorphic physical characteristics such as brain size and 2D:4D. The typical male brain is heavier than the female brain (Harden et al., 2001) which is a difference

that may in part be due to early FT exposure (Hines, 2004). In addition, as discussed in Chapter 2, results from studies of 2D:4D ratio show that children with autism have lower (i.e. hyper-masculinised) digit ratios compared to typically developing boys. These patterns have also been observed in the siblings and parents of children with autism, suggesting genetically-based elevated FT levels may have a role in the development of autism (Manning et al., 2001; Milne et al., 2006). However, as mentioned in Chapter 5, a study using a large Austrian sample of adults found no significant relationships between 2D:4D ratio and the AQ-Adult (Voracek & Dressler, 2006).

Investigation of individuals with Congenital Adrenal Hyperplasia (CAH) found that girls with this condition exhibit more autistic traits, measured using the AQ-Adult compared to unaffected control females (Knickmeyer et al., 2006a). Whilst CAH provides an interesting opportunity to investigate the effects of additional androgen exposure, the relatively rare occurrence of CAH in conjunction with ASC makes it difficult to obtain large enough sample sizes for generalisation of research findings to the wider population. However, researchers have suggested that CAH-related disease characteristics, rather than prenatal androgen exposure, could be responsible for the atypical cognitive profiles found in this population (Fausto-Sterling, 1992; Quadagno et al., 1977).

6.1.2. *Aims*

The aim of this study is to examine the possibility of a link between prenatal exposure to testosterone and the development of autistic traits in children between 6-10 years of age. Evaluation of autistic traits is completed by using the CAST and a modified version of the Autism Spectrum Quotient for children (AQ-Child). Although the CAST was specifically developed to report ASC in children, it was designed as a screening tool and may be less useful for measuring the range of autistic traits in research studies indicated by previous findings of skewed distributions (Williams et al., 2005). The AQ has been developed as a measure of different characteristics of behaviour associated with ASC in

a wider population of adults and adolescents, resulting in a more normal distribution of scores (Baron-Cohen et al., 2006a; Baron-Cohen et al., 2001a).

In the first part of this chapter, the AQ was modified for children. Study 1 reports the development of a new parent-report measure of autistic traits in children, the Autism Spectrum Quotient–Children's Version (AQ-Child). The AQ-Child was adapted from the AQ-Adult. The psychometric properties of the AQ-Child were investigated by administration to n=1225 typically developing and n=540 children with an ASC diagnosis. Data from these samples were also analysed to identify an appropriate cut-off score that may predict the presence of ASC. Scoring patterns in both childhood and adulthood were analysed to investigate if similar scoring patterns are found in children and adults. The development of the Children's version of the Autism Spectrum Quotient (AQ-Child) allows for longitudinal comparison and could be a useful measure for following the development of autistic traits.

In the second part of this chapter (Study 2), the CAST and newly developed AQ-Child were administered to parents of children participating in the Cambridge FT Project. The aim of Study 2 is to examine the relationship between autistic traits and FT exposure in this sample of typically developing children.

6.2. Study 1: Development of the AQ-Child

6.2.1. *Study 1 Method*

6.2.1.1. The AQ-Child

The AQ-Child is a 50-item measure developed to detect autistic traits in children between 4 and 11 years of age. The AQ-Child was designed to be a parent-report questionnaire, since self-report by children might be restricted by reading and comprehension difficulties. It was adapted from the adult and adolescent versions of the AQ, and items that were not age-appropriate in the adult questionnaires were revised

accordingly. Items in the AQ-Child were kept as close to the AQ-Adult and AQ-Adol as possible, with most questions aimed at the same behaviours (see Appendix 6). Items were worded to produce an approximately equal agree/disagree response in order to avoid a response bias.

The AQ-Child consists of a series of descriptive statements designed to assess five areas associated with autism and the broader phenotype: social skills (items 1, 11, 13, 15, 22, 36, 44, 45, 47, 48), attention switching (items 2, 4, 10, 16, 25, 32, 34, 37, 43, 46), attention to detail (items 5, 6, 9, 12, 19, 23, 28, 29, 30, 49), communication (items 7, 17, 18, 26, 27, 31, 33, 35, 38, 39) and imagination (items 3, 8, 14, 20, 21, 24, 40, 41, 42, 50), each represented by ten items. Higher scores correspond to more 'autistic-like' behaviour.

In the scoring system of Baron-Cohen et al. (2001) items are scored as one for a response in the 'autistic' direction and zero for a 'non-autistic' response. The response scale in the present study adopted a scoring scheme used in recent studies of the AQ-Adult (Austin, 2005; Hoekstra et al., 2007) where the response scale is treated as a four-point Likert scale. Parents rate to what extent they agree or disagree with the statements about their child, with the following answer categories: 0=definitely agree, 1=slightly agree, 2=slightly disagree and 3=definitely disagree. Items were reverse scored as necessary. This method was used because it was anticipated that the degree of endorsement of each item contained additional information and could be useful for studies of autistic traits. Total AQ scores were represented by the sum of each item score. The minimum AQ score (zero) indicates no autistic traits; the maximum score (150) suggests full endorsement on all autistic items.

6.2.1.2. Study 1 Participants

Group 1 (n=1225, 618 girls, 607 boys) included children who were participating in a large epidemiological study of social and communication skills in children at ages 4 to 9 (Scott et al., 2002b; Williams et al., 2005). Participants were excluded if the child had any

of the following conditions: ASC (n=34), language delay (n=58), dyspraxia (n=13), epilepsy (n=3), or Attention-deficit hyperactivity disorder (n=28). Twin births (n=32) or siblings (n=62) of children with an ASC were also excluded. In the case where more than one child in a family was participating, the child whose age was closest to the mean age of the control group (M=9.82, SD=1.27) was retained, and the other siblings were excluded (n=48). Siblings and twin births were excluded to ensure independence of data. Initially, 2777 questionnaires were sent out by post, resulting in a response rate of approximately 50%. This sample was drawn from primary schools in Cambridgeshire, UK.

Group 2 comprised children diagnosed with an ASC by psychiatrists using established DSM-IV criteria (APA, 1994). Children with a diagnosis of autism (n=192, mean age=7.58 (SD=2.43)) or Asperger syndrome/High-Functioning autism (AS/HFA) (n=348) were included in the study, mean age=9.31, (SD=2.10). Children with a diagnosis of Pervasive Developmental Disorder - Not Otherwise Specified (PDD/NOS) (n=26) or atypical autism (n=4) were excluded from the study due to the small sample sizes. Children with a diagnosis of autism were grouped separately from the children with an AS/HFA diagnosis. Mothers of these children were recruited via the University of Cambridge Autism Research Centre website and completed the questionnaires online (www.autismsresearchcentre.com).

Questionnaires with more than five blank items were considered incomplete and these data were discarded in subsequent analyses (n=57). If five or fewer answers were missing, the AQ-Child score was corrected for missing items by making the following calculation: total AQ-Child score + (mean item score × number of missing items) (Hoekstra et al., 2007). This was performed for n=9 participants.

6.2.2. *Study 1 Results*

6.2.2.1. Item Analysis

An item analysis was conducted to examine scoring patterns on each item (see Table 6.1). Inspection of these scores showed that there were three items where controls scored higher than children with an ASC diagnosis (items 29, 30, 49). All three of these items focus on 'attention to detail'. Closer inspection suggests that these items may be difficult to examine for young children and so these were eliminated in subsequent analyses.

Table 6.1. Item Analysis – Mean scores for each item by each group

Item	Subdomain	Controls (n=1225)	AS/HFA (n=348)	Autism (n=191)
AQ1	S	0.89	1.89	2.08
AQ2	A	1.06	2.48	2.50
AQ3	I	0.61	1.47	1.99
AQ4	A	1.75	2.77	2.68
AQ5	D	1.23	2.51	2.31
AQ6	D	1.38	2.20	1.98
AQ7	C	0.45	2.41	2.51
AQ8	I	0.46	1.70	2.16
AQ9	D	0.82	1.43	1.09
AQ10	A	0.97	2.63	2.76
AQ11	S	0.70	2.69	2.61
AQ12	D	1.78	2.52	2.36
AQ13	S	0.34	1.48	1.32
AQ14	I	0.74	1.79	2.30
AQ15	S	0.93	2.21	2.18
AQ16	A	1.57	2.74	2.59
AQ17	C	0.58	2.33	2.49
AQ18	C	1.49	2.30	1.53
AQ19	D	1.10	1.70	1.48
AQ20	I	0.59	2.20	2.32
AQ21	I	0.46	1.50	1.54
AQ22	S	0.60	2.50	2.45
AQ23	D	1.10	2.05	1.87
AQ24	I	0.83	1.45	1.56
AQ25	A	0.80	2.32	2.22
AQ26	C	0.42	2.43	2.57
AQ27	C	1.08	2.66	2.68
AQ28	D	1.25	2.40	2.51
AQ29	D	1.86	1.72	1.23

AQ30	D	2.02	1.67	1.77
AQ31	C	1.07	2.62	2.64
AQ32	A	0.75	2.46	2.32
AQ33	C	0.54	2.20	2.26
AQ34	A	0.55	2.18	1.87
AQ35	C	0.80	2.24	2.46
AQ36	S	0.74	2.49	2.38
AQ37	A	0.70	2.18	1.96
AQ38	C	0.57	2.63	2.75
AQ39	C	1.18	2.61	1.97
AQ40	I	0.67	2.43	2.63
AQ41	I	1.18	2.17	1.52
AQ42	I	0.97	2.40	2.39
AQ43	A	1.39	1.85	1.46
AQ44	S	0.32	1.90	1.72
AQ45	S	1.10	2.61	2.58
AQ46	A	1.36	2.64	2.43
AQ47	S	0.69	1.93	1.88
AQ48	S	0.64	2.35	2.45
AQ49	D	1.69	1.36	1.04
AQ50	I	0.48	2.12	2.42

Key: C=Communication, S=Social Skills, A=Attention Switching, D=Attention to Detail, I=Imagination.

6.2.2.2. Internal Consistency

Cronbach's α coefficients were calculated and for the measure as a whole, the α coefficient was high (α=0.97) for both sexes combined as well as in girls (α=0.94) and boys (α=0.97).

6.2.2.3. Test-retest reliability

A random selection of 500 parents were asked to complete a second copy of the AQ-Child to examine test-retest reliability, resulting in 272 test-retest pairs (141 girls, 131 boys). The mean time interval between the first and second test was 12.3 weeks (SD=2.01). For the AQ-Child, the correlation between the two tests was good ($r=0.85$, $p<0.001$).

6.2.2.4. Factor analysis of the AQ-Child

The retained 47 items of the AQ-Child were subjected to principal components analysis (PCA). Prior to performing PCA, the suitability of the control AQ-Child data for factor analysis was assessed. Inspection of the correlation matrix revealed the presence of many coefficients of 0.30 and above. Diagnostic checks suggested that the data were suitable for analysis: the Kaiser-Meyer-Oklin value was 0.93, and the Bartlett's Test of Sphericity reached statistical significance (χ^2 =19841.29, df=1081, p<0.001).

An oblique rotation was used, since it is reasonable to assume that the sub-components would be related. Five components arose explaining 21.8%, 8.9%, 5.1%, 4.2% and 3.2% of the variance. An inspection of the scree plot revealed a clear break after the first, second and fourth components. It was decided to retain four components for further investigation. Before rotation, the four-component solution explained a total of 40% of the variance. All items with factor pattern matrix elements greater than 0.3 are included. The factors were named Mind-reading, Attention to Detail, Social Skills and Imagination. These factors were found to be respectively highly correlated with the original AQ sub-scales of Communication, Attention to Detail, Social Skills, and Imagination (see Table 6.2).

Table 6.2. Correlations between new and original AQ subscales

New Subscale	Original Subscale	Correlation	Sig.
Mind-reading	Communication	.97	p<0.001
Attention to Detail	Attention to Detail	.95	p<0.001
Social Skills	Social Skills	.97	p<0.001
Imagination	Imagination	.97	p<0.001

Table 6.3 presents the items of the four scales in order of loadings on the components (highest first). The eigen values of the rotated factors and the percentages explained by

each of the factors are also shown. The internal reliabilities of the new factors were 0.96, 0.85, 0.94 and 0.90 respectively.

Table 6.3. Factor Structure of the AQ-Child

Item	Content	Loading
	Mindreading - Cronbach's α=0.96, eigen value=6.73, % variance=21.77	
39	Keeps going on and on about the same thing.	0.663
45	Finds it difficult to work out people's intentions.	0.590
18	Doesn't let others to get a word in edgeways.	0.577
35	Often the last to understand a joke.	0.555
31	Knows how to tell if someone bored.	0.539
37	Can switch back after an interruption.	0.529
4	Gets strongly absorbed in one thing.	0.484
27	Finds it easy to "read between the lines".	0.468
36	Finds it easy to work out feelings by looking at faces.	0.455
2	Prefers to do things the same way.	0.454
7	Is impolite, even though s/he thinks it is polite.	0.410
10	Can easily keep track of several conversations.	0.402
32	Finds it easy to do more than one thing at once.	0.376
42	Finds it difficult to imagine being someone else.	0.376
33	Doesn't know when it's their turn on the phone.	0.355
48	Is a good diplomat.	0.331
	Attention to Detail - Cronbach's α=0.85, eigen value=4.73, %variance=8.85	
6	Notices numbers or strings of information.	0.783
23	Notices patterns.	0.735
9	Fascinated by dates.	0.714
19	Fascinated by numbers.	0.700
12	Notices details that others do not.	0.690
5	Notices small sounds when others do not.	0.548
41	Likes to collect information.	0.529
43	Likes to plan activities carefully.	0.436
16	Tends to have very strong interests.	0.421
	Social Skills - Cronbach's α=0.94, eigen value=7.59, %variance=5.12	
44	Enjoys social occasions.	-0.777
38	Good at social chit-chat.	-0.744
47	Enjoys meeting new people.	-0.707
17	Enoys social chit-chat.	-0.694
11	Finds social situations easy.	-0.636
22	Finds it hard to make new friends.	-0.635
1	Prefers to do things with others	-0.533
15	Finds it hard to make new friends.	-0.515
26	Doesn't know how to keep up a conversation.	-0.510
13	Would rather go to a library than a party.	-0.503
46	New situations make him/her anxious.	-0.422
34	Enjoys doing things spontaneously.	-0.405
25	Gets upset when daily routine is disturbed.	-0.294
24	Would rather go to the theatre than the library	-0.279
28	Concentrates on the whole picture rather than details	-0.222

	Imagination - Cronbach's α=0.90, eigen value=4.80, %variance=4.20	
14	Finds making up stories easy.	0.751
8	Can easily imagine what story characters look like.	0.698
3	Finds it very easy to create a mental picture.	0.654
21	Doesn't particularly enjoy reading fiction.	0.548
50	Finds it to easy to play games that involve pretending.	0.511
20	Finds it difficult to work out the characters' intentions in a story.	0.480
40	Enjoyed playing games involving pretending.	0.447

6.2.2.5. Group differences

Group differences were examined using both the original AQ-Child subscales and on scores for the factors discussed above. Mean AQ-Child scores (total) for each group, broken down by sex and by subscale are shown in Table 6.4.

Table 6.4. Mean scores for subscales and total by Group

		AQ Total	Communi-cation	Attention to Detail	Social Skills	Imagi-nation	Attention Switching	Factor1-Mindreading	Factor 2-Atten. Detail	Factor3-Social Skills	Factor4-Imagination
Controls (n=1225)	x	41.7	8.2	8.7	7.0	7.0	10.9	15.3	11.6	10.8	4.0
	SD	18.6	5.0	4.5	5.0	4.6	5.1	7.9	5.7	7.4	3.7
Control boys (n=607)	x	45.7	9.0	8.9	7.8	8.5	11.5	16.6	12.0	12.0	5.1
	SD	20.0	5.4	4.7	5.3	4.9	5.6	8.5	6.0	7.8	4.0
Control girls (n=618)	x	37.7	7.4	8.5	6.1	5.5	10.3	14.0	11.1	9.7	2.9
	SD	16.1	4.4	4.4	4.6	3.7	4.6	7.1	5.4	6.7	3.1
AS/HFA (n=348)	x	104.8	24.4	14.7	22.1	19.2	24.2	39.4	19.2	33.0	13.2
	SD	15.6	4.0	4.0	5.1	5.4	4.2	5.7	4.9	7.0	4.7
AS/HFA boys (n=312)	x	104.8	24.4	14.9	21.9	19.4	24.2	39.3	19.4	32.8	13.3
	SD	15.7	4.0	4.0	5.1	5.2	4.2	5.6	4.9	7.1	4.6
AS/HFA girls (n=36)	x	104.7	24.9	13.7	23.4	17.9	24.7	40.2	17.7	34.6	12.2
	SD	15.7	4.2	3.5	4.4	6.7	3.6	5.8	4.3	6.1	5.7
Autism (n=192)	x	103.0	23.9	13.7	21.7	20.9	10.9	38.1	16.7	32.7	15.4
	SD	16.3	4.1	4.4	5.1	5.4	22.8	5.9	5.5	7.2	4.2
Autism boys (n=156)	x	103.6	24.0	13.7	21.7	21.3	22.9	38.2	16.9	32.9	15.7
	SD	15.1	3.9	4.4	4.8	4.7	4.2	5.7	5.5	6.5	4.0
Autism girls (n=36)	x	100.2	23.5	13.3	21.8	19.2	22.4	38.1	15.9	32.0	14.2
	SD	20.8	5.0	4.8	6.2	6.2	5.3	6.6	5.9	9.6	4.7
Controls vs. AS/HFA	t	57.89**	56.02**	22.92**	49.17**	42.27**	44.62**	53.08**	22.68**	59.99**	38.25**
Controls vs. Autism	t	43.26**	41.71**	14.22**	37.72**	38.61**	30.59**	38.35**	11.69**	38.47**	38.75**
AS/HFA vs. Autism	t	1.25	1.47	3.12*	0.75	3.51**	3.70**	2.47*	5.35**	0.37	5.42**

*p<.01, **p<.001

Examination of the original subscales using an ANOVA of total AQ-Child score by group (control, AS/HFA and autism) and sex showed a significant effect of group ($F_{2, 1759}$=1277.66, p<0.001). Post Hoc Dunnett T3 tests revealed that the two clinical groups scored significantly higher than the typically developing group (p<0.001), but that the two clinical groups did not differ from each other. The main effect of sex was also significant ($F_{1, 1759}$=6.33, p=0.01). The interaction between group and sex was also significant ($F_{2, 1759}$=3.56, p<0.05).

T-tests confirmed that there was a significant sex difference (t(1154.11)=7.02, p<0.001, equal variances not assumed) in the control group (males scoring higher than females), confirming the same effect reported with the AQ-Adult and AQ-Adol. There were no significant sex differences in the clinical groups (Autism group: t(190)=0.26, p>0.05; AS/HFA group: t(346)=0.15, p>0.05). See Figure 6.1 for a visual representation of AQ-Child distribution scores for control girls and boys and for the AS/HFA and Autism groups. The clinical groups differed from the control group on all subdomain scores (for t-tests results see Table 6.4). No association between age and AQ Total was found (r=0.03, p>0.05) suggesting that AQ scores are independent of age in this sample.

Figure 6.1. Scoring patterns on the AQ-Child by group

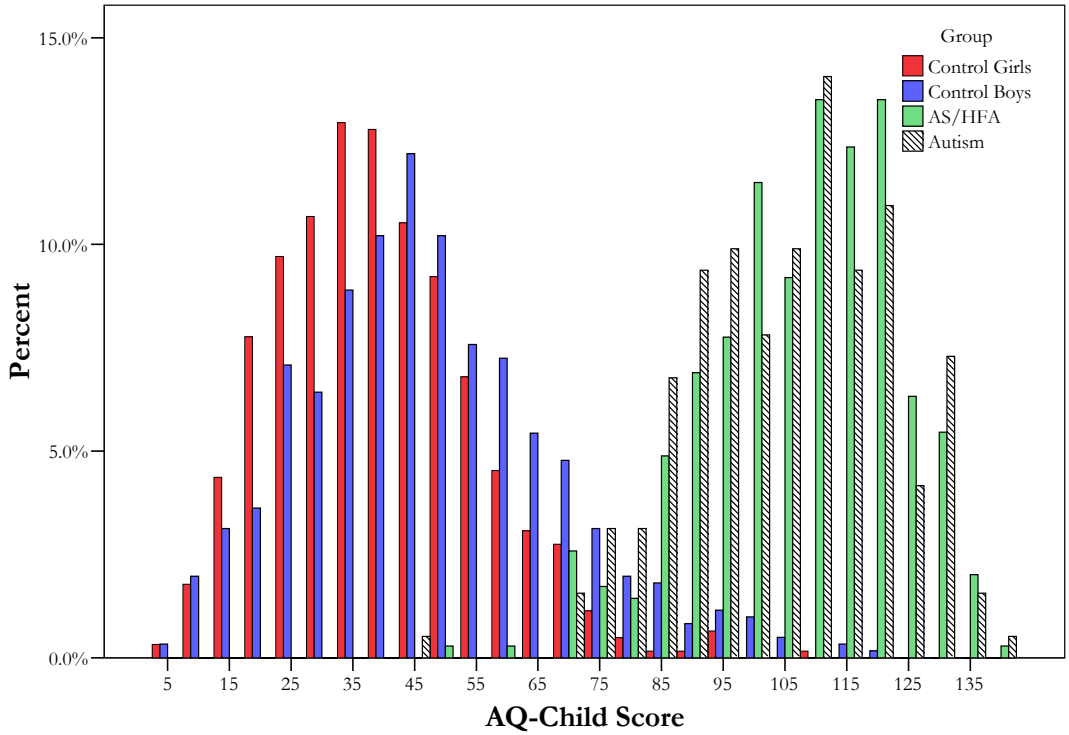
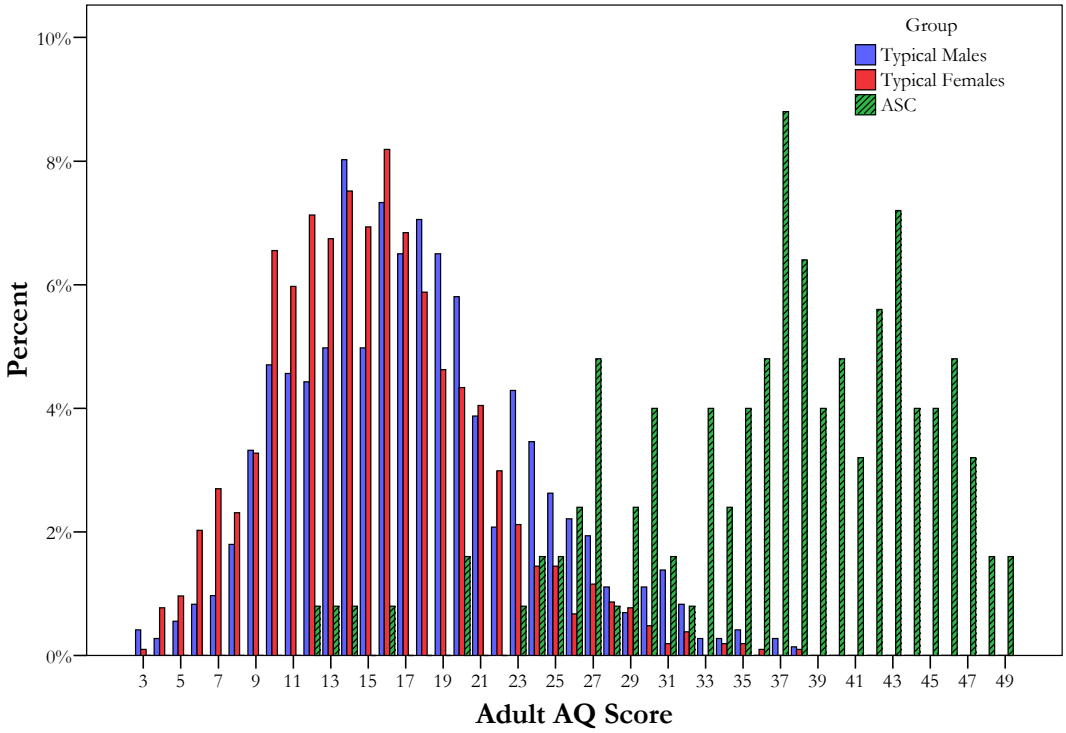


Figure 6.2. Scoring patterns on the Adult AQ by group

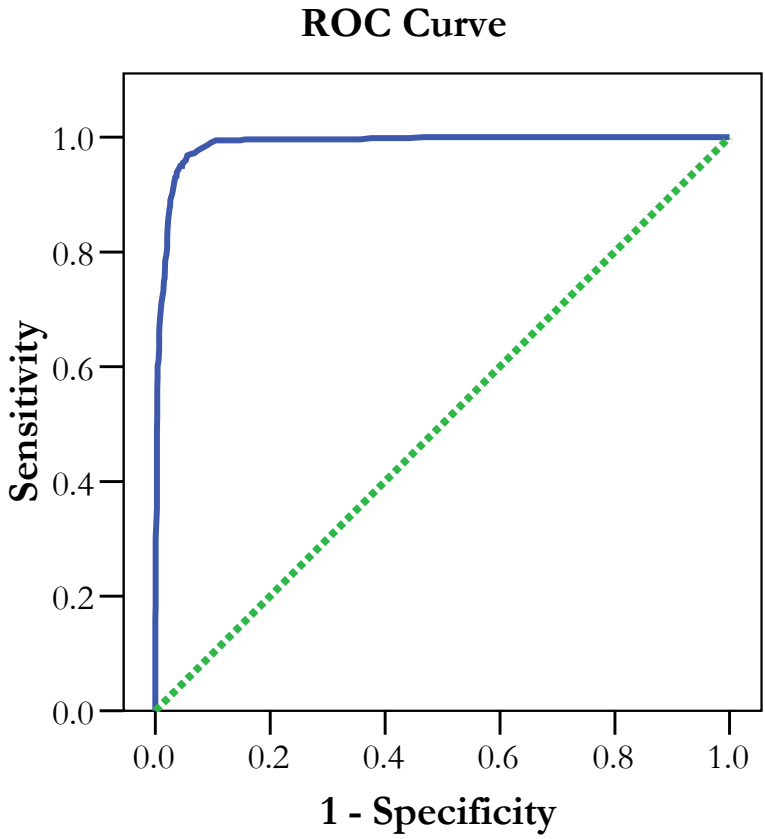


Data from: Wheelwright, S., Baron-Cohen, S., Goldenfeld, N., Delaney, J., Fine, D., Smith, R., et al. (2006). Predicting Autism Spectrum Quotient (AQ) from the Systemising Quotient-Revised (SQ-R) and Empathy Quotient (EQ). Brain Research, 1079, 47-56.

6.2.2.6. Cut-off scores

Figure 6.2 shows the Receiver-Operating-Characteristic (ROC) curve. The area under the ROC curve was 0.99 (95% C.I.: 0.98 to 0.99), indicating that total score is a good indicator of ASC diagnosis.

Figure 6.3. ROC curve of the sensitivity and specificity of AQ-Child Score



Note: Area under the curve = 0.99

Table 6.5 also shows sensitivity and specificity values for a range of potential cut-offs as well as the percentage of each group (by sex) scoring at or above each cut-off (maximum obtainable score=141 when only using retained items). Adult (Baron-Cohen et al., 2001a) and adolescent (Baron-Cohen et al., 2006a) data are also shown. The ROC analysis showed that a score of 76 showed both high sensitivity (95%) as well as high specificity (95%). With a cut-off of 76, less than 2% of control girls and 7% of control

boys scored at or above the ASC cut-off, whereas 95% of children with AS/HFA and 95% of children with Autism scored at or above this cut-off.

For the AQ-Adult, a score of 32 or above was chosen as the cut-off since 79.3% of the clinical group scored at or above this score, whereas only 2% of the control adults did so (Baron-Cohen et al., 2001a). The AQ-Adol used a cut-off of 30 (Baron-Cohen et al., 2006a).

Table 6.5. Comparison of AQ cut-off points for various ages

	AQ-Child Cut-off points				AQ-Adult Cut-off		AQ-Adol Cut-off	
Indices	n	66	76	86	n	32	n	30
Sensitivity	-	0.99	0.95	0.86	-	-	-	-
Specificity	-	0.90	0.95	0.98	-	-	-	-
% Controls scoring above cut-off	1225	9.7	4.3	2.2	174	2.3	50	0.0
% Control Females	618	4.7	1.6	1.0	98	1.0	25	0.0
% Control Males	607	14.8	7.1	3.5	76	3.9	25	0.0
% AS/HFA scoring above cut-off	348	98.9	95.1	87.1	58	79.3	52	90.4
% AS/HFA Females	36	97.2	94.4	94.4	13	92.3	14	92.3
% AS/HFA Males	312	99.0	95.2	86.2	45	75.6	38	89.5
% Autism scoring above cut-off	192	99.5	94.8	82.8	-	-	79	88.6
% Autism Females	36	97.2	86.1	80.6	-	-	16	81.3
% Autism Males	156	100.0	96.8	83.3	-	-	63	90.5

*Adult data from: Baron-Cohen, S., Hoekstra, R., Knickmeyer, R., & Wheelwright, S. (2006). The Autism-Spectrum Quotient (AQ) - Adolescent version. *Journal of Autism and Developmental Disorders*, *36*, 343-350.
*Adolescent data from: Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E. (2001). The Autism Spectrum Quotient (AQ) : Evidence from Asperger syndrome/high functioning autism, males and females, scientists and mathematicians. *Journal of Autism and Developmental Disorders*, *31*, 5-17.

6.3. Study 2: Foetal testosterone and autistic traits

6.3.1. *Study 2 Method*

6.3.1.1. Study 2 Participants

The AQ-Child and CAST were sent to all mothers (n=456) meeting inclusion criteria, 261 mothers completed the CAST and 248 mothers completed the AQ-Child, resulting

in a total of 235 (118 boys, 117 girls) children with complete data for both questionnaires.

6.3.2. *Outcome variables*

The Child Autism Spectrum Quotient (AQ-Child). This is the measure developed in Study 1 to detect autistic traits in children 4-11 years of age (maximum score=150). Higher scores indicate a greater number of autistic traits. A score of 76+ indicates a risk for ASC. AQ-Child items are answered in a Likert format (definitely agree, slightly agree, slightly disagree and definitely disagree). The AQ-Child has shown good test-retest reliability ($r=0.85$, $p<0.001$), high sensitivity (95%) and high specificity (95%). Principal components analysis suggests the AQ-Child has four empirically derived subscales: mind-reading, attention to detail, social skills and imagination, with a maximum attainable score of 141.

The Childhood Autism Spectrum Test (CAST) (Scott et al., 2002b). This 37-item parent-report questionnaire was developed to detect ASC in 4-11 year-old children (Scott et al., 2002b). CAST items require a binary response ('yes/no') to 37 questions, 31 of which are scored (maximum score of 31). A validation study suggested that a score of 15 or above should be used to indicate risk for ASC (Scott et al., 2002b; Williams et al., 2005). The CAST has good test-retest reliability, good positive predictive value (50%) and high specificity (97%) and sensitivity (100%) for ASC (Williams et al., 2005).

6.3.3. *Study 2 Results*

Table 6.6 presents the means and standard deviations for each sex separately, as well as combined for predictor variables, CAST and AQ-Child scores. Table 6.7 shows the correlation coefficients for both the predictor and outcome variables. Tables 6.8 and 6.9 show correlation coefficients for girls and boys separately.

Examination of the univariate distributions revealed that FT level was positively skewed, and was the only predictor variable with a distribution that deviated significantly from the Gaussian distribution. Four female outliers in FT levels (individuals who scored three or more standard deviations from the mean) were observed. These outlying values were replaced using a windsorizing procedure, where the extreme values were replaced by the highest observed level within three standard deviations from the mean (0.80 nmol/L). Windsorized FT levels showed no outliers and acceptable skewness statistics for both boys and girls, and were used in subsequent analyses. No significant sex differences were found for any of the predictor variables except FT level.

Table 6.6. Descriptive statistics for Study 2

	Combined Group				Girls				Boys				
Variable	n	M	SD	Range	n	M	SD	Range	n	M	SD	range	Cohen's d
^FT level (nmol/L)**	235	0.60	0.44	0.05-2.30	117	0.35	0.31	0.05-2.30	118	0.85	0.41	0.10-2.05	1.38
Gestational Age	162	16.49	1.44	13-22	81	16.57	1.48	14-22	81	16.40	1.40	13-20	0.12
Child Age	217	8.91	0.95	6.97-10.68	109	8.80	0.97	7.01-10-68	108	9.02	0.92	6.97-10.66	0.23
Maternal Age	210	35.77	4.40	23.68-45.90	106	35.88	4.22	23.68-45.66	104	35.66	4.59	24.28-45.90	0.05
Parental Education	207	3.24	1.01	1-5	102	3.18	0.87	2-5	105	3.30	1.14	1-5	0.12
^CAST Total*	235	4.65	3.87	0-22	117	4.08	3.24	0-18	118	5.22	4.35	0-22	0.30
AQ-Child Total**	235	41.62	18.02	6-103	117	34.42	15.01	6-80	118	48.75	17.96	16-103	0.87

^Indicates raw values

* Sex difference significant at the $p<0.05$ level

** Sex difference significant at the $p<0.01$ level

Table 6.7. Correlation matrix for all cases

	FT Level	Sex	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	CAST Score
Sex	.63**	--	--	--	--	--	--	--	--
Gestational Age	.04	.06	--	--	--	--	--	--	--
Child Age	.03	.12	-.05	--	--	--	--	--	--
Maternal Age	-.02	-.03	-.28**	-.06	--	--	--	--	--
Parent Education	.07	.06	-.10	-.05	.16*	--	--	--	--
Older Sister	-.07	-.08	-.11	-.10	-.03	-.07	--	--	--
Older Brother	-.04	-.12	-.10	.01	.09	-.15*	.36**	--	--
CAST Score	.25**	.14*	.03	.08	-.13	-.02	-.01	-.07	--
AQ-Child Score	.41**	.40**	.01	-.01	-.01	-.06	-.19**	-.14*	.25**

* $p<0.05$, ** $p<0.01$

Table 6.8. Correlation matrix for Girls

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	CAST Score
Gestational Age	-.01	--	--	--	--	--	--	--
Child Age	.03	-.07	--	--	--	--	--	--
Maternal Age	-.01	-.36**	-.13	--	--	--	--	--
Parent Education	.03	.02	-.06	.12	--	--	--	--
Older Sister	-.03	-.02	-.02	-.20*	-.17	--	--	--
Older Brother	-.06	-.14	.03	.07	-.22*	.37**	--	--
CAST Score	.08	-.02	-.01	-.09	-.06	-.11	-.21*	--
AQ-Child Score	.27**	.01	-.11	.10	-.14	-.17	-.23*	.31**

* $p<0.05$, ** $p<0.01$

Table 6.9. Correlation matrix for Boys

	FT Level	Gest. Age	Child Age	Matr. Age	Parent Education	Older Sister	Older Brother	CAST Score
Gestational Age	-.01	--	--	--	--	--	--	--
Child Age	-.11	-.01	--	--	--	--	--	--
Maternal Age	.00	-.22	.01	--	--	--	--	--
Parent Education	.05	-.18	-.05	.19	--	--	--	--
Older Sister	-.03	-.22	-.18	.16	.03	--	--	--
Older Brother	.14	-.05	.03	.11	-.08	.32**	--	--
CAST Score	.28**	.07	.12	-.15	-.02	.10	.13	--
AQ-Child Score	.23*	.06	-.02	-.09	-.06	-.18	.05	.15

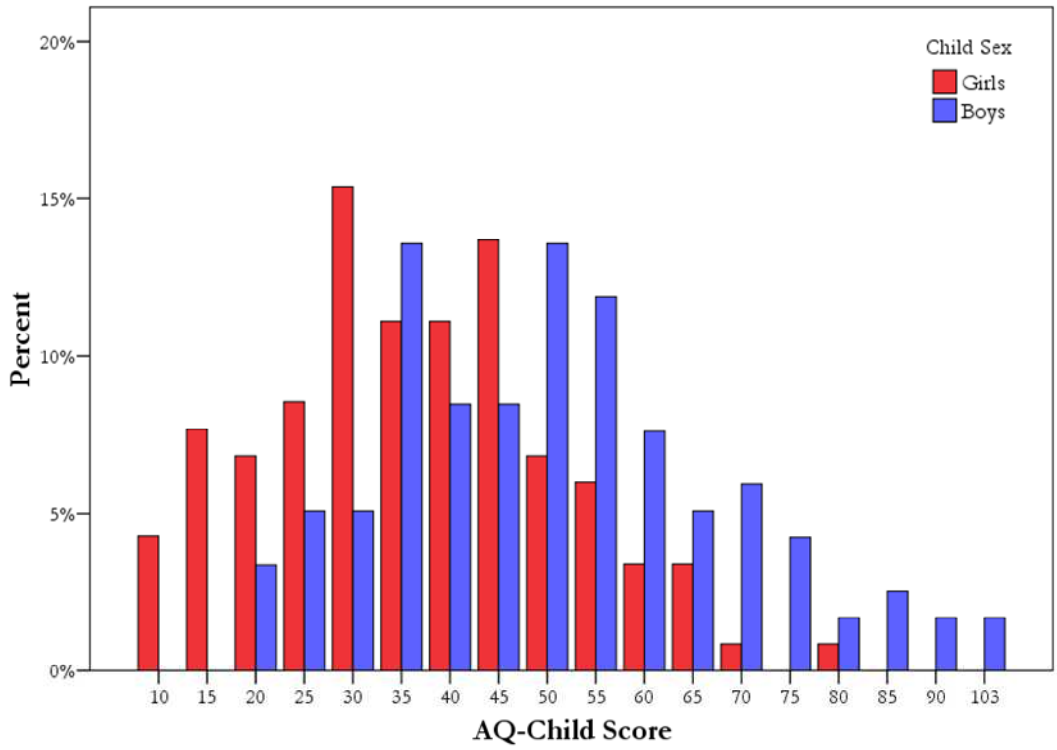
* $p<0.05$, ** $p<0.01$

6.3.4. *AQ-Child Scores*

AQ-Child Total. Scores on the AQ-Child showed significant sex-differences, t(233)=6.64, p<0.001 (equal variances assumed), with boys (M=48.75, SD=17.96) scoring higher than girls (M=34.42, SD=15.01).

For AQ-Child total score, examination of the univariate distribution revealed that it was not skewed (skewness<1) for all cases together as well as in boys and girls separately. Figure 6.4 shows the raw distribution of total AQ-Child scores. Raw AQ-Child scores were used in subsequent analyses.

Figure 6.4. Distribution of AQ-Child scores



6.3.4.1. Internal Consistency

Cronbach's α was calculated for the AQ-Child measure and demonstrated good internal consistency for the sexes together (α=0.89) and for girls (α=0.86) and boys (α=0.89)

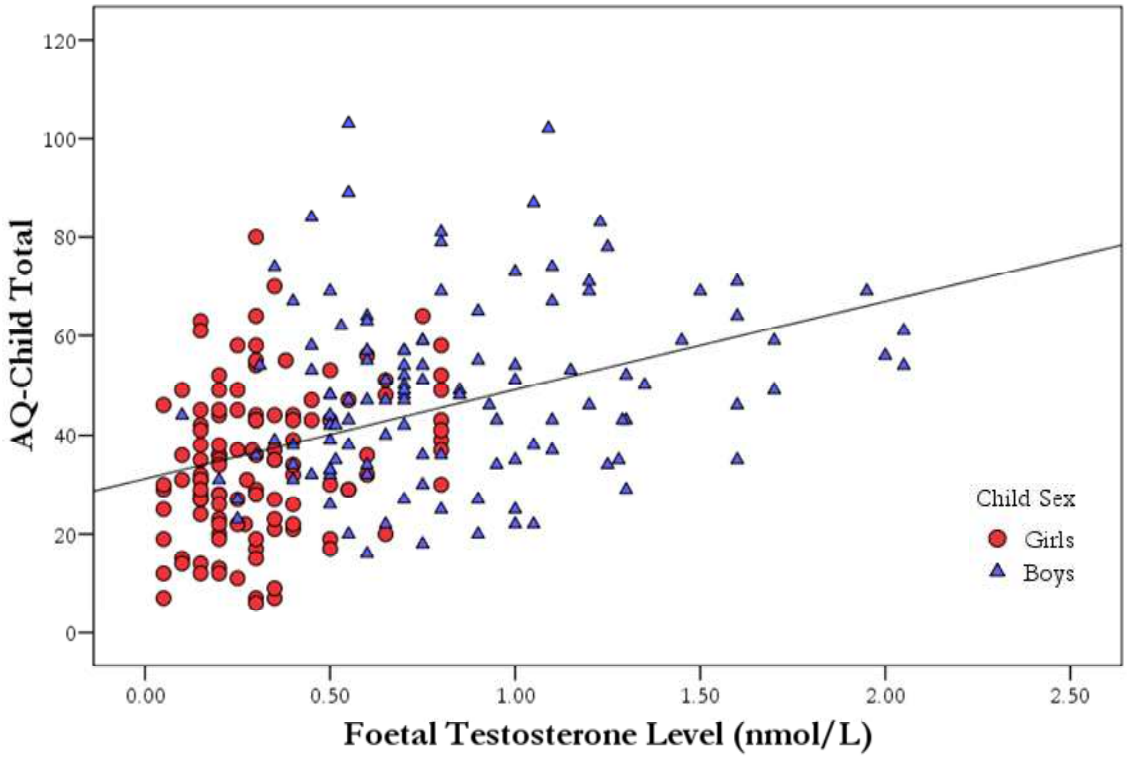
separately. Split half reliability was good for the entire sample (0.86) and for girls (0.79) and boys (0.87).

Table 6.10. Final Regression Model for AQ-Child scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
AQ-Child Total	Older Sister	0.04	0.04	7.36	3.45	0.13*	p<0.05
	Older Brother			3.53	4.03	0.06	p>0.05
	FT level	0.20	0.16	11.61	3.23	0.27**	p<0.01
	Sex	0.23	0.03	3.82	1.35	0.21**	p<0.01
		Girls only					
AQ-Child Total	Parent education	0.06	0.06	3.28	1.60	0.20	p<0.05
	Older sisters			4.23	3.95	0.11	p>0.05
	Older brothers			6.91	4.50	0.16	p>0.05
	FT level	0.15	0.08	22.30	7.22	0.29	p<0.01
		Boys only					
AQ-Child Total	Older sisters	0.05	0.05	12.54	5.84	0.20	p<0.05
	Older brothers			7.42	7.79	0.09	p>0.05
	FT level	0.09	0.05	8.91	3.93	0.21	p<0.05

For the hierarchical regression analysis, the predictor variables that correlated with total AQ-Child scores at $p<0.20$ were presence of older sisters ($r=-0.19$, $p<0.01$) and presence of older brothers ($r=-0.14$, $p<0.05$). The inclusion of FT level in the second block produced a significant F-change ($F=46.35$, $p<0.001$, $\Delta R^2=0.16$). Inclusion of Sex in the final regression model also produced a significant F-change (F-change=7.99, $p<0.05$, $\Delta R^2=0.03$). The Sex/FT level interaction was excluded as a predictor from the final regression model (see Table 6.10). Figure 6.5 shows a visual representation of the relationship between FT level and AQ-Child scores for males and females combined.

Figure 6.5. Relationship between FT level and AQ-Child scores



Within sex analyses were conducted to further investigate scoring patterns in boys and girls separately. For girls only, parent education level ($r=-0.14$, $p<0.01$), presence of older sisters ($r=-0.17$, $p<0.01$) and older brothers ($r=-0.23$, $p<0.05$) showed correlations at the $p<0.20$ level and were entered into the first block using the enter method. FT level ($r=0.27$, $p<0.001$) was tested for entry into the regression model in the second block. A significant F-change (F-change=4.12, $p<0.01$, $\Delta R^2=0.08$) was observed when FT was entered into the regression in the second block. The predictor variables that correlated with AQ-Child scores at the $p<0.20$ level for boys were presence of older sisters ($r=-0.19$, $p<0.01$) and brothers ($r=-0.14$, $p<0.05$). Presence of older sisters and brothers were included in the first block using the enter method. FT level ($r=0.22$, $p<0.001$) was tested for entry in the second block using the stepwise method. The final model included FT level, which showed a significant F-change (F-change=5.13, $p<0.05$, $\Delta R^2=0.05$). Residual analysis revealed acceptable plots and no outliers.

AQ-Child Subscales. A mean score was calculated for each subscale due to the uneven number of items, allowing for comparisons between the subscales. Sex differences were

explored among the four empirical AQ-Child subscales (see Table 6.11). All four subscales showed significant sex differences (all $p<0.001$) with boys scoring higher than girls.

Table 6.11. Examination of AQ-Child subscale scores by sex

	Girls (n=117)		Boys (n=118)		
Variable	M	SD	M	SD	t
Mindreading	0.83	0.46	1.09	0.50	4.14**
Attention to Detail	1.05	0.56	1.37	0.58	4.26**
Social Skills	0.58	0.39	0.89	0.50	5.30**
Imagination	0.43	0.43	0.82	0.53	6.15**
AQ-Child Total	34.42	15.01	48.75	17.96	6.64**

* $p<0.05$
** $p<0.01$

Mindreading, Attention to Detail, Social Skills and Imagination were significantly associated with FT level and Sex. These results are consistent with those observed in AQ-Child Total when all participants are examined together (see Table 6.12). The internal consistency of the AQ-Child subscales were also satisfactory (Mindreading=0.80; Attention to Detail=0.80; Social Skills=0.87; and Imagination=0.75).

Table 6.12. Correlations for FT level and AQ-Child Subscales

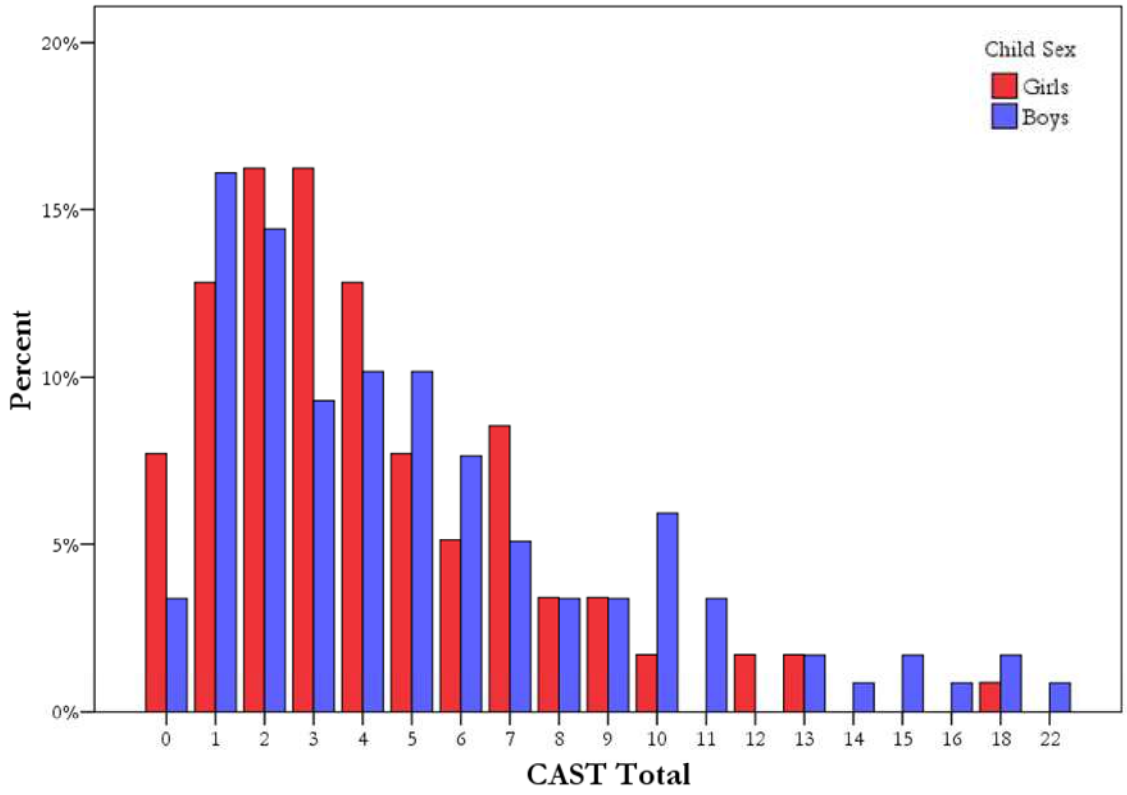
	FT Level	Mind-reading	Attn. Detail	Social Skills	Imagi-nation
Mindreading	.30**	--	--	--	--
Attention to Detail	.27**	.35**	--	--	--
Social Skills	.33**	.62**	.31**	--	--
Imagination	.38**	.38**	.17**	.38**	--
AQ-Child Total	.41**	.86**	.61*	.83**	.57**

6.3.5. *CAST Scores*

Examination of univariate distributions indicated that the distribution for CAST scores was positively skewed. Figure 6.6 shows the raw distribution of CAST scores. CAST

scores were transformed by adding one and taking the square root of each score, resulting in a distribution that was not significantly skewed.

Figure 6.6. Distribution of raw CAST scores



Transformed CAST scores showed significant sex-differences, $t(226.55)=2.12$, $p<0.05$, equal variances not assumed, with boys ($M=2.36$, $SD=0.82$) scoring higher than girls ($M=2.15$, $SD=0.69$).

6.3.5.1. Internal Consistency

Cronbach's α for the CAST measure showed satisfactory internal consistency for all cases (α=0.85) and for girls (α=0.74) and boys (α=0.89) separately. Split half reliability was good for the sexes together (0.85), and in girls (0.71) and boys (α=0.90) separately.

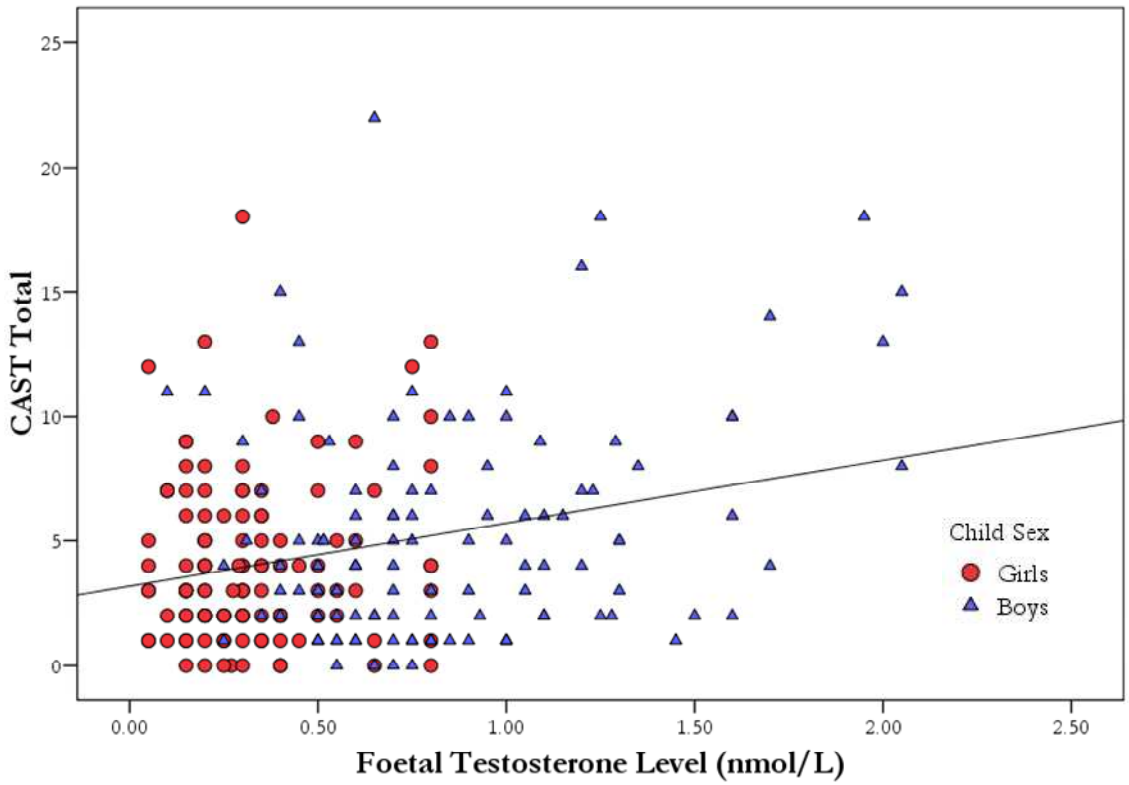
Table 6.13. Final Regression Model for CAST scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
CAST Total	Mother Age	0.02	0.02	0.02	0.01	0.13	p>0.05
	FT level	0.07	0.05	11.61	3.23	0.22	p<0.01
		Girls only					
CAST Total	No significant predictors						
		Boys only					
CAST Total	Mother Age	0.06	0.06	0.03	0.02	0.18	p>0.05
	Older brothers			0.34	0.27	0.13	p>0.05
	Older sisters			0.32	0.35	0.09	p>0.05
	FT level	0.12	0.06	0.50	0.20	0.25	p=0.01

Note: Square-root transformation was conducted before analysis

For the regression analysis, the predictor variables that correlated with CAST scores at the p<0.20 level, were Sex (r=0.14, p<0.05), FT (r=0.25, p<0.001) and maternal age (r=-0.13, p=0.06). No suppressor variables were observed. Inclusion of FT level in the second block produced a significant F-change (F-change=10.72, p<0.01, R^2=0.07). The main effect of sex was excluded as a predictor. Residual analysis showed no outliers and acceptable plots (see Table 6.13). See Figure 6.7 for a visual representation of the relationship between FT level and CAST scores.

Figure 6.7. Relationship between FT level and CAST scores



In addition, to further investigate whether the results might be due to a sex difference (not necessarily involving FT), the relationship between these scores and FT within each sex was examined. For boys, maternal age ($r=-0.15$, $p=0.12$) and presence of older brothers ($r=0.14$, $p=0.15$) met criteria for entry into the analysis ($r=0.14$, $p<0.001$). Presence of older sisters was included as a suppressor variable due to its high correlation with the presence of older brothers ($r=0.32$, $p<0.001$). Inclusion of FT level in the second block produced a significant F-change (F-change=6.57, $p<0.05$, $R^2=0.12$). For girls alone, no significant relationship was found between CAST scores and FT levels, therefore regression analyses were not conducted.

CAST scores were significantly correlated with AQ-Child scores when boys and girls were examined together ($r=0.25$, $p<0.001$) and when girls were examined alone ($r=0.31$, $p=0.001$). However, these measures were not significantly correlated in boys ($r=0.15$, $p>0.05$).

6.4. Discussion

Study 1 examined the psychometric properties of the Autism Spectrum Quotient-Children's version (AQ-Child). The AQ-Child showed good test-retest reliability and high internal consistency for the questionnaire as a whole as well as for each of the original five sub-scales (Communication, Attention to Detail, Social Skills, Imagination and Attention Switching). Principal Components Analysis provided support for four factors which were highly correlated with four of the original subscales: Communication (renamed Mind-reading), Attention to Detail, Social Skills and Imagination. These four factors also showed high reliability coefficients.

A cut-off of 76 was adopted and showed high sensitivity (0.95) and specificity (0.95). These results demonstrate that the AQ-Child has good construct validity since individuals with a diagnosis score significantly higher than those without a diagnosis (Figure 6.1). However, caution should be exercised when using any cut-off to indicate diagnosis because this is not dependent on an absolute score but on whether the traits cause impairments in everyday functioning (APA, 1994; ICD-10, 1994). In addition, no confirmation has been obtained that individuals in Group 1 with no clinical diagnosis do not have an ASC, and future population-based studies could assess those who score above the cut-off to see whether those without a diagnosis would warrant one.

Sex differences in AQ-Child score were found in the control group, with typically developing boys scoring higher than girls. Similar results are reported in many measures of ASC. No sex differences were found in the AS/HFA or autism group, but there were also many more boys (n=312) in the clinical group than girls (n=36). This is a common problem since the high male to female ratio of ASC limits the number of females available to participate in research studies. It would be beneficial for future research to obtain larger samples that can more closely examine scoring patterns between males and females with ASC.

Within the clinical group (Group 2), comparisons between the AS/HFA and autism group showed no significant difference in total AQ-Child score. However, several items in this questionnaire (e.g. 10, 27, 33) concern behaviours that may require some conversational competence, and it is possible that the addition of these items prevented a complete measure of autistic traits in subgroups of ASC such as classic autism. As a result, the AQ-Child is most useful for individuals with some speech and with intelligence in the borderline average range (70) or above. It would be interesting for future researchers to examine the relationship between AQ-Child score and severity of symptoms, since this was outside the scope of this study.

Age was not associated with AQ-Child score in this study. These results are consistent with those obtained from the Adolescent and Adult versions of the AQ (Baron-Cohen et al., 2006a; Baron-Cohen et al., 2001a), and a comparison between AQ scores in children, adolescents and adults reveals very similar scoring patterns. The current results suggest that the AQ-Child measures traits that are consistent throughout the age groups. It would be useful in future studies to test the correlation between the AQ-Child and related measures that have been used in primary school age children and to validate the AQ-Child in a large population sample.

Results from Study 1 suggest that the AQ-Child is a strong measure which can discriminate the presence of ASC in a clinical sample. The AQ-Child could also be useful as a measure of the broader autism phenotype in epidemiological samples. The strong similarity in scoring patterns for children and adults are consistent with the idea that autistic traits develop early in life and are persistent.

Study 2 directly investigated the relationship between FT levels and the later development of autistic traits (as measured by the Childhood Autism Spectrum Test (CAST) and the AQ-Child). FT levels were found to be positively associated with number of autistic traits. The significant positive relationship between FT levels was observed across CAST total score, AQ-Child total score, as well as in the four subscales

of the AQ-Child. Results remained consistent when excluding individuals who score above the established cut-offs for the CAST and AQ-Child.

Scores from the AQ-Child showed a significant positive relationship with FT levels when the sexes were combined as well as when they were examined independently. The CAST, however, was found to have a significant positive relationship with FT levels when the sexes were combined and in boys only. No relationship was found between CAST and FT levels when girls were examined alone. It is of interest to note that most other research examining the role of testosterone in human psychosexual development has produced more supportive evidence in females than in males (Hines, 2004; Hines, 2006). The variation of CAST scores measured in girls was much smaller than in boys for this study, possibly accounting for the lack of any effect.

The correlation between CAST and AQ-Child scores ($r=0.25$, $p<0.001$) was statistically significant but the low coefficient did not suggest high convergent validity. FT levels predicted about 7% of the variation in CAST scores, and 20% of the variation in AQ-Child scores. These differences might be accounted for by the structure of each questionnaire. The CAST was developed as a screening tool and more participants in the general population achieve a low score. This design may also account for the negatively skewed score distribution of the CAST. The AQ-Child was designed to measure autistic traits in the wider population and is a more graduated measure of the exhibition of a particular trait. It would be useful for future research to examine the sensitivity and specificity of both these instruments using a large sample of high scorers to ascertain how well these measures (using established cut-off scores) identify those at risk for ASC.

Although the two measures of autistic traits reported in this study show limited correlations with each other, both measures report higher scores in boys. Results from both measures also suggest a role for FT level in the development of autistic traits providing support for the Extreme Male Brain (EMB) theory at both psychometric and

biological levels. While similar findings have been reported previously using *indirect* biological measures such as the 2D:4D ratio (assumed to be a proxy for FT) (Manning et al., 2001), or brain activity using fMRI (Baron-Cohen et al., 2006b), this is the first time that such a relationship has been reported using *direct* measures. We can assume that the observed positive association between FT levels and autistic traits may reflect a direct causal effect of FT on neural development, but this remains speculative due to the correlational design of this study. It is for example possible that FT is serving as an index for an unknown third variable.

Findings from Study 2 provide additional support that variations in FT levels are related to autistic traits in typically developing children, but results should be extrapolated with caution to individuals with a formal diagnosis of ASC. The current study is too small a sample to be able to test if FT levels are elevated in formally diagnosed cases of ASC, since these have a prevalence rate of about 1% (Baird et al., 2006). A further study is planned which will involve a large-scale collaboration so as to increase sample size sufficiently to compare FT levels in sufficient numbers of cases vs. controls.

Evidence from previous chapters suggests the possibility of a link between prenatal exposure to testosterone and masculinisation of certain sexually dimorphic behaviours. Other evidence supports the characterisation of ASC as an extreme manifestation of certain male-typical behaviours. In this chapter, the AQ-Child is developed in order to evaluate the possibility that elevated FT levels are a risk factor for autistic traits. The psychometric properties of this measure of autistic traits was examined in a large sample of typically developing children and a further sample with a diagnosis of ASC in Study 1. Study 2 investigated the possibility of a link between FT levels and autistic traits using the AQ-Child and the CAST, which has been validated in a series of previous studies as a screening tool for ASC. Sex differences were found for both measures, with boys scoring higher than girls. FT levels were positively associated with higher scores on the CAST and AQ-Child. Within sex analyses showed this link to be stronger in girls than boys for the AQ-Child. For CAST scores, no significant predictors were found in girls.

However, the CAST has been designed as a binary response screening tool. Since ASC are much rarer in girls, a correlation might be expected in a larger sample size. Together, results from Study 1 and 2 provide further support for the EMB theory of autism and suggest a role for foetal androgens in the development of characteristics associated with ASC.

Chapter 7: Autistic traits in toddlers

In addition to the foetal surge of testosterone that is thought to occur during the second trimester of pregnancy, another neonatal testosterone (NT) surge has been observed which takes place shortly after birth and lasts for approximately 3-4 months. The oestrogen hormone oestradiol is another hormone which forms from prenatal testosterone. In this chapter, the links between autistic traits, FT levels, foetal oestradiol (FO) levels and neonatal testosterone (NT) levels were investigated. Since NT has not been explored previously, a new cohort of children was established to examine this effect. Study 1 reports an investigation of the relationship between FT levels and autistic traits in n=129 toddlers, and revealed that FT levels were positively associated to autistic traits, measured using the Quantitative Checklist for Autism in Toddlers (Q-CHAT). Study 2 examines the relationships between FT levels, FO levels, NT levels and autistic traits in a subset of children (n=35) from Study 1. No relationships between FO, NT levels and Q-CHAT scores were observed and showed no sex differences and no relationships with FT levels. A relationship between FT and Q-CHAT was observed in this subset of children when the sexes were combined but not within sex, possibility because of the smaller sample sizes. Sex differences were also observed in Q-CHAT scores in both Studies 1 and 2. These studies provide further support for the EMB theory of autism and suggest that whilst FT level is associated with higher Q-CHAT scores, this effect does not extend to NT or to FO levels.

7.1. Introduction

Studies presented in previous chapters have evaluated the possibility of a link between FT levels, sex-typical traits and attributes of the EMB theory of autism. However, as discussed in Chapter 1, there is also thought to be a second peak in testosterone levels in early life, occurring from just after birth and throughout the first few months of life (Smail et al., 1981). In addition to testosterone, the oestrogen hormone oestradiol is another hormone which forms prenatally from testosterone. Oestradiol is considered to be the most biologically active oestrogen (Collaer & Hines, 1995). Studies in rodents suggests that it masculinises the brain when it is synthesised via aromatisation of testosterone and related precursors (Collaer & Hines, 1995). The role of oestradiol in the development of male-typical behaviours in humans is less certain, since studies have not shown significant associations with the development of later behaviour (Knickmeyer et al., 2005a; Knickmeyer et al., 2006b; Knickmeyer et al., 2005b; Lutchmaya et al., 2002a; Lutchmaya et al., 2002b; van de Beek et al., 2004; van de Beek et al., 2008). Conversely, a significant negative association between the ratio of foetal testosterone (FT) and foetal oestradiol (FO) levels and right hand 2D:4D ratio has been observed (Lutchmaya et al., 2004).

In this chapter, the links between autistic traits, FT levels, FO levels and neonatal testosterone (NT) levels were investigated. Since NT samples have not been examined previously in the Cambridge FT Project, a new cohort of children was established to investigate this effect.

7.1.1. *Aims*

In Study 1 of this chapter, a new cohort of participants was recruited in order to establish whether autistic traits measured in toddlers are related to prenatal levels of testosterone and oestradiol. Measurements of autistic traits were made using the Quantitative Checklist for Autism in Toddlers (Q-CHAT). Although it is widely recognised that autism has a prenatal onset (Volkmar, Stier & Cohen, 1985), current

methods of diagnosis are stable from about 2 to 3 years of age (Baron-Cohen et al., 1996; Lord, 1995; Lord et al., 2006). Recent studies have, however, suggested that characteristics of ASC are recognised by parents of children as young as 18 to 24 months of age (Allison et al., 2008; Baron-Cohen, Allen & Gillberg, 1992; De Giacomo & Fombonne, 1998).

Study 2 was a preliminary investigation of the relationships between FT, FO and NT levels and autistic traits in a small subset of children from Study 1. Mothers from Study 1 were asked to bring their 3 month-old infants to Addenbrooke's Hospital for the purposes of collecting saliva samples which were assayed for testosterone levels, with the goal of measuring whether foetal and/or neonatal testosterone levels show any relationships with Q-CHAT scores.

7.2. Study 1: FT and Q-Chat

7.2.1. *Study 1 Methods*

7.2.1.1. Birth Cohort 2 Participants

This study utilised a sample of children from Birth Cohort 2. Participants were excluded if: (a) amniocentesis revealed a chromosomal abnormality; (b) the pregnancy ended in miscarriage or termination; (c) the child suffered neonatal or infant death; (d) the child suffered significant medical problems after birth; (e) there was a twin pregnancy or (f) the relevant information was absent from medical records. Questionnaires were sent to all mothers whose General Practitioner gave consent. These women were contacted and asked for permission to analyse their amniotic fluid for foetal testosterone levels, resulting in 283 mothers contacted for participation in the current series of studies. 135 mothers completed the Q-CHAT, however, 6 participants left 10 or more items blank and their data were considered incomplete. The final sample for this study included 129 participants (66 boys, 63 girls) with complete data.

7.2.1.2. Outcome variable

Quantitative Checklist for Autism in Toddlers (Q-CHAT) (Allison et al., 2008). This is a 25-item parent-report screening measure developed to identify toddlers at risk for the development of ASC. This measure is a major revision of the Checklist for Autism in Toddlers (CHAT) which was a screening tool originally developed for use by Health professionals at around 18 months of age (Baron-Cohen et al., 1992). The Q-CHAT allows for a larger range of response categories, where the items are scored on a 5-point scale. Sex differences have been observed for this measure, where boys (M=27.50, SD=7.80) scored higher than girls (M=25.80, SD=7.70) in large population sample (Allison et al., 2008).

7.2.2. *Predictor variables*

FT levels. The predictor of greatest interest in Study 1 is FT (see Chapter 2 for a detailed description).

Foetal Oestrogen (FO) levels (pmol/L). Amniotic oestradiol levels were assayed by the Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge. Amniotic fluid was extracted with diethyl ether. Recovery experiments have demonstrated consistent 95% recovery of oestradiol using this method. The oestradiol was measured by fluorescence-labelled immunoassay. The Wallac-Delfia method was used (Wallac OY, Turku, Finland). This assay uses a polyclonal rabbit antibody to oestradiol in a competitive format in which sample oestradiol competes with europium-labelled oestradiol analogue for the antibody binding sites. A second antibody directed against rabbit IgG is coated to the microtitre plate and is used to capture the first antibody and its bound oestradiol analogue. After washing, the europium is measured by time-resolved fluorescence. Calibration is with pure 17beta-estradiol. The detection limit is 25 pmol/L. The cross reactivity with steroids other than 17beta oestradiol is very low. It should be noted that 16 hydroxy and 16 oxo-steroids, steroids that are formed in the foeto-placental unit, cross react to less than 0.9% by weight. Intra-assay coefficients of

variation (i.e. 1 standard deviation expressed as a percentage of the mean value) were 5.2% at 180 pmol/L and 3.9% at 875 pmol/L.

The control variables included in this study were the same as those described in Chapter 2: gestational age, maternal age, parental education level, presence of older brothers and sisters and child age.

7.2.3. *Study 1 Results*

Examination of the univariate distributions revealed that FT and FO levels were positively skewed, and were the only predictor variables with a distribution that deviated significantly from the Gaussian distribution. Three male outliers in FT levels (individuals who scored three or more standard deviations from the mean) were observed. These outlying values were replaced using a windsorizing procedure, where the extreme values are replaced by the highest observed level within three standard deviations from the mean (1.55 nmol/L). No outliers were found when girls' FT levels were examined. Windsorized FT levels showed no outliers and acceptable skewness statistics for both boys and girls, and are used in subsequent analyses. Levels of FO were positively skewed (skewness>1) so a natural logarithmic transformation was carried out. This reduced the skewness considerably (skewness<1), and transformed data were used in subsequent analyses.

Table 7.1 presents the means and standard deviations for each sex separately, as well as combined for predictor variables, and Q-CHAT scores. Table 7.2 shows the correlation coefficients for predictor and outcome variables for all cases. Tables 7.3 and 7.4 show correlation coefficients for girls and boys separately.

Table 7.1. Descriptive Statistics for Study 1

	Combined Group				Girls				Boys				
Variable	n	M	SD	Range	n	M	SD	Range	n	M	SD	range	Cohen's D
^FT level (nmol/L)**	129	0.59	0.41	0.05-2.28	63	0.34	0.27	0.05-1.12	66	0.82	0.42	0.15-2.28	1.36
^FO level (pmol/L)	129	307.12	186.29	108-1260	63	309.67	181.98	126-1260	66	304.70	191.68	108-1220	0.03
Gestational Age	120	16.92	1.83	13-26	60	17.01	1.62	15-22.3	60	16.83	2.03	13-26	0.10
Child Age	129	20.36	4.33	17-39	63	20.10	3.23	17-36	66	20.61	5.18	18-39	0.12
Maternal Age	129	35.67	4.21	21-46	63	35.81	4.26	24-46	66	35.53	4.18	21-45	0.07
Parental Education	127	3.56	1.05	1-5	61	3.58	1.05	1-5	66	3.54	1.06	1-5	0.04
Q-CHAT Score**	129	26.55	7.08	10-43	63	24.94	6.52	10-43	66	28.09	7.30	14-43	0.46

^Indicates raw values

* Sex difference is significant at the $p<0.05$ level

** Sex difference is significant at the $p<0.01$ level

Table 7.2. Correlation matrix for all cases

	FT Level	FO Level	Sex	Gest. Age	Child Age	Matr. Age	Parent Ed	Older Sister	Older Brother
FO Level	.22*	--	--	--	--	--	--	--	--
Sex	.61**	-.02	--	--	--	--	--	--	--
Gestational Age	-.01	-.15	-.05	--	--	--	--	--	--
Child Age	-.07	.04	.06	.21*	--	--	--	--	--
Maternal Age	-.02	-.08	-.03	-.21*	-.22*	--	--	--	--
Parent Education	-.17	-.09	-.06	-.06	-.12	-.01	--	--	--
Older Sister	-.03	.01	-.10	.00	.03	.12	-.19*	--	--
Older Brother	.01	-.13	.08	-.11	-.06	.05	.01	-.12	--
Q-CHAT Score	.40**	.01	.22*	-.13	-.08	.06	-.02	-.07	.07

* $p<0.05$, ** $p<0.01$

Table 7.3. Correlation matrix showing relationships between variables for Girls

	FT Level	FO Level	Gest. Age	Child Age	Matr. Age	Parent Ed	Older Sister	Older Brother
FO Level	.17	--	--	--	--	--	--	--
Gestational Age	.26*	-.22	--	--	--	--	--	--
Child Age	.01	.06	.24	--	--	--	--	--
Maternal Age	-.03	-.13	-.27*	-.20	--	--	--	--
Parent Education	-.07	.04	-.05	-.07	-.02	--	--	--
Older Sister	-.17	-.06	-.10	.00	.06	-.11	--	--
Older Brother	.01	.06	.05	.28*	-.18	-.07	-.17	--
Q-CHAT Score	.31*	.06	.06	.08	.09	.12	-.07	.10

* $p<0.05$, ** $p<0.01$

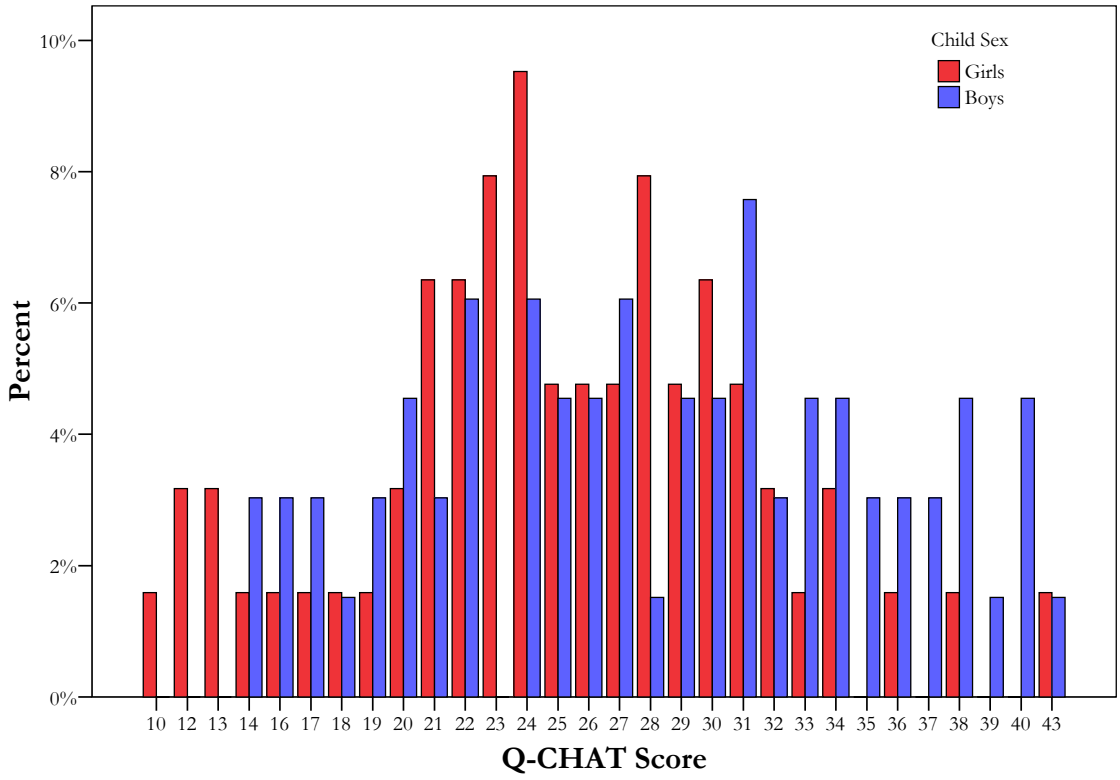
Table 7.4. Correlation matrix showing relationships between variables for Boys

	FT Level	FO Level	Gest. Age	Child Age	Matr. Age	Parent Ed	Older Sister	Older Brother
FO Level	.37**	--	--	--	--	--	--	--
Gestational Age	-.10	-.09	--	--	--	--	--	--
Child Age	-.18	.03	.20	--	--	--	--	--
Maternal Age	.01	-.03	-.18	-.24	--	--	--	--
Parent Education	-.25*	-.25*	-.09	-.15	-.02	--	--	--
Older Sister	.18	.09	.09	.06	.17	-.31*	--	--
Older Brother	-.08	-.31*	-.23	-.26*	.27*	.10	-.06	--
Q-CHAT Score	.36**	-.01	-.25	-.19	.05	-.13	-.03	.03

* $p<0.05$, ** $p<0.01$

Examination of the univariate distribution of Q-CHAT scores revealed that it was not skewed (skewness<1) for all cases together as well as in boys and girls separately, and raw scores were used in further analyses. Figure 7.1 shows the raw distribution of Q-CHAT scores.

Figure 7.1. Distribution of Q-CHAT Scores



Independent samples t-tests showed significant sex differences in FT levels $t(110.93)=8.71$, $p<0.001$ (equal variances not assumed), with boys ($M=0.80$, $SD=0.36$) showing higher levels than girls ($M=0.34$, $SD=0.23$). No sex differences were observed in FO levels $t(127)=0.22$, $p>0.05$. When the sexes were combined, a significant relationship between FT level and sex was observed ($r=0.61$, $p<0.001$). Whilst a significant association was found between FT levels and FO levels ($r=0.22$, $p<0.05$), no significant link was found between Sex and FO levels ($r=-0.02$, $p>0.05$).

Scores on the Q-CHAT showed significant sex-differences, $t(233)=6.64$, $p<0.001$ (equal variances assumed), with boys ($M=48.75$, $SD=17.96$) scoring higher than girls

(M=34.42, SD=15.01). No significant sex differences were observed for any of the control variables (all p>0.05).

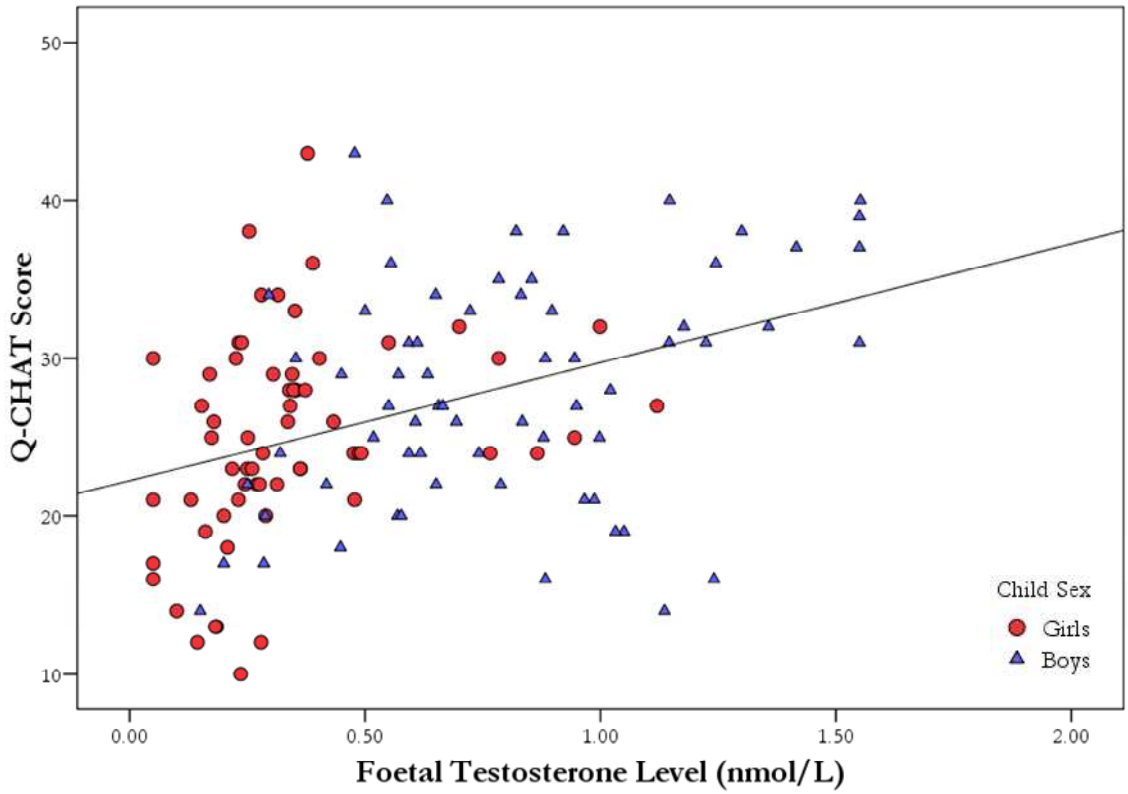
The internal consistency was calculated for the Q-CHAT and the Cronbach's α was adequate (α=0.55) for the sexes combined and for boys and girls separately. The internal consistency for the entire measure was sufficient for girls (α=0.49) and boys (α=0.56) separately. Split-half reliability was also adequate (0.59) for the sample as a whole, for girls (0.47) and for boys (0.65).

Table 7.5. Final Regression Model for Q-CHAT scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
Q-CHAT Score	Gestational age	0.02	0.02	0.47	0.32	0.12	p>0.05
	FT level	0.18	0.16	7.32	1.53	0.40	p<0.001
		Girls only					
Q-CHAT Score	FT level	0.10	0.10	8.97	3.50	0.31	p<0.05
		Boys only					
Q-CHAT Score	Gestational age	0.08	0.08	0.71	0.43	0.20	p>0.05
	Child age			0.10	0.17	0.07	p>0.05
	FT level	0.21	0.13	7.19	2.38	0.37	p<0.01

For the hierarchical regression analysis of Q-CHAT scores presented in Table 7.5, the variable included at the first stage using the enter method was gestational age (r=-0.13, p<0.20). FT level (r=0.40, p<0.001) and Sex (r=0.22, p<0.05) were included in the second stage using the stepwise method. The FT/Sex interaction was also tested for inclusion at the third stage using the stepwise method. The second stage retained FT levels (F-change=22.78, p<0.001, ΔR^2=0.16), while sex and the FT/Sex interaction were excluded from the final model.

Figure 7.2. Relationship between FT level and Q-CHAT scores



For girls alone, no variables met criteria for entry into the hierarchical regression analyses except for FT level ($r=0.31$, $p<0.05$). This was entered at the first stage using the stepwise method. FT level was retained and produced a significant F-change (F-change=6.59, $p<0.05$, $\Delta R^2=0.10$). For boys, gestational age ($r=-0.25$, $p<0.20$) and child age ($r=-0.19$, $p<0.20$) were entered at the first stage of the regression analysis. Inclusion of FT level at the second stage also produced a significant F-change (F-change=9.14, $p<0.01$, $\Delta R^2=0.13$).

7.3. Study 2: Neonatal testosterone and Q-CHAT: A pilot study

7.3.1. *Study 2 Methods*

7.3.1.1. Study 2 Participants

Mothers who consented to participate in Study 1 were contacted and asked to participate in this study of neonatal hormone levels. A total of 47 (22 boys, 25 girls) mothers brought their children in for saliva collection at Addenbrooke's Hospital where a sample of passive drool was taken when their child reached 3-months of age. However, 12 samples had insufficient amounts of saliva to measure testosterone levels, with a total of 35 samples (15 boys, 20 girls) remaining that were eligible for the testosterone assay. The saliva samples were collected using a suction machine under the supervision of a paediatrician. The families were contacted when their child reached 18 months, and were asked to complete The Quantitative Checklist for Autism in Toddlers (Q-CHAT).

7.3.1.2. Predictor Variables

Neonatal Testosterone (NT) levels. Saliva samples were assayed (without separation or extraction) for testosterone using commercially available immunoassay protocols (Salimetrics, State College, PA) using an EIA with a lower limit of sensitivity of 1.5 pg/ml, and average intra- and inter-assay coefficients of variation less than 10% and 15%, respectively. A serial dilution was performed (2.5) to give a standard curve with greater sensitivity at lower ranges. As such, the standard curve used for this assay ranged from 240, 96, 38.4, 15.4, 6.1, 2.44, 0 pg/mL. Units of salivary testosterone are expressed in picograms per millilitre (pg/mL).

In addition to NT levels, FT and FO level measurements were also included in the analyses. The same control variables from previous studies were used in the current sample.

7.3.2. *Study 2 Results*

Salivary (or neonatal) testosterone levels were not skewed, and therefore raw values were used. Windsorized FT levels and transformed oestradiol levels were utilised since these distributions approached that of the Gaussian distribution. The distributions of the control variables also did not differ from those of the larger sample, and were not skewed. Q-CHAT scores were also not skewed for this subset of children; therefore raw Q-CHAT scores were used in subsequent analyses. Due to the small sample sizes, nonparametric statistics were used to analyse the data.

Table 7.6 presents the means and standard deviations for each sex separately, as well as combined for predictor variables, and Q-CHAT scores. Table 7.7 shows the correlation coefficients for predictor and outcome variables for all cases. Tables 7.8 and 7.9 show correlation coefficients for girls and boys separately.

Table 7.6. Descriptive Statistics for Study 2

	Combined Group				Girls				Boys				
Variable	n	M	SD	Range	n	M	SD	Range	n	M	SD	range	Cohen's D
NT level (pg/mL)	35	49.92	17.37	4.36-84.29	20	52.16	19.10	4.36-84.29	15	46.93	14.88	11.33-65.55	0.31
FT level (nmol/L)*	35	0.58	0.45	0.05-2.28	20	0.34	0.23	0.05-0.95	15	0.91	0.47	0.42-2.28	1.54
Oestradiol level (pmol/L)*	35	278.77	179.19	145-1150	20	235.50	82.06	145-449	15	336.47	250.20	175-1150	0.54
Gestational Age	35	16.70	1.47	13-21.2	19	16.62	0.90	15-18.1	14	16.82	2.04	13-21.2	0.13
Child Age	35	19.34	2.93	18-35	20	19.10	1.33	18-23	15	19.67	4.27	18-35	0.18
Maternal Age	35	36.54	4.74	21-46	20	37.75	3.84	33-46	15	34.93	5.44	21-45	0.60
Parental Education	35	3.41	0.90	1.5-5	20	3.30	0.89	1.5-4.5	15	3.57	0.92	2-5	0.30
Q-CHAT Score**	35	27.03	8.62	10-43	20	23.95	8.62	10-43	15	31.13	6.94	14-40	0.92

*Indicates raw values

* Sex difference is significant at the $p<0.05$ level

** Sex difference is significant at the $p<0.01$ level

Table 7.7. Correlation matrix for all cases

	NT Level	FT Level	FO Level	Sex	Gest. Age	Child Age	Matr. Age	Parent Ed	Older Sister	Older Brother
FT Level	.13	--	--	--	--	--	--	--	--	--
FO Level	-.11	.03	--	--	--	--	--	--	--	--
Sex	-.13	.72**	.28	--	--	--	--	--	--	--
Gestational Age	.10	.17	-.47**	.04	--	--	--	--	--	--
Child Age	-.21	-.08	.22	-.09	-.10	--	--	--	--	--
Maternal Age	-.18	-.40*	-.09	-.28	.10	.10	--	--	--	--
Parent Education	.31	.07	.12	.13	-.09	-.03	-.02	--	--	--
Older Sister	-.09	-.12	-.18	-.14	.02	.38*	.32	-.05	--	--
Older Brother	-.31	-.08	.05	.02	-.10	.18	-.04	.19	.03	--
Q-CHAT Score	-.09	.56**	.17	.46**	-.10	.18	-.32	.06	-.08	.15

* $p<0.05$, ** $p<0.01$

Table 7.8. Correlation matrix showing relationships between variables for Girls

	NT Level	FT Level	FO Level	Gest. Age	Child Age	Matr Age	Parent Ed	Older Sister	Older Brother
FT Level	.22	--	--	--	--	--	--	--	--
FO Level	-.40	-.32	--	--	--	--	--	--	--
Gestational Age	.46*	.45	-.50*	--	--	--	--	--	--
Child Age	-.30	.20	.41	-.29	--	--	--	--	--
Maternal Age	-.41	-.14	.13	.00	.13	--	--	--	--
Parent Education	.63**	.14	.12	.26	.08	-.17	--	--	--
Older Sister	-.31	.25	-.13	.02	.39	.10	-.14	--	--
Older Brother	-.21	.09	.31	-.28	.24	-.23	.22	-.07	--
Q-CHAT Score	-.01	.57**	.09	-.21	.35	-.03	.12	.12	.33

* $p<0.05$, ** $p<0.01$

Table 7.9. Correlation matrix showing relationships between variables for Boys

	NT Level	FT Level	FO Level	Gest. Age	Child Age	Matr. Age	Parent Ed	Older Sister	Older Brother
FT Level	.43	--	--	--	--	--	--	--	--
FO Level	.44	.10	--	--	--	--	--	--	--
Gestational Age	-.27	-.19	-.55*	--	--	--	--	--	--
Child Age	-.10	-.22	-.04	.21	--	--	--	--	--
Maternal Age	-.04	-.44	-.20	.31	-.03	--	--	--	--
Parent Education	-.15	-.13	-.07	-.37	-.13	.29	--	--	--
Older Sister	.27	-.50	-.16	.10	.26	.59*	.19	--	--
Older Brother	-.42	-.38	-.39	.08	.12	.25	.13	.21	--
Q-CHAT Score	-.09	.51	-.23	.04	-.13	-.39	-.19	-.27	-.05

* $p<0.05$, ** $p<0.01$

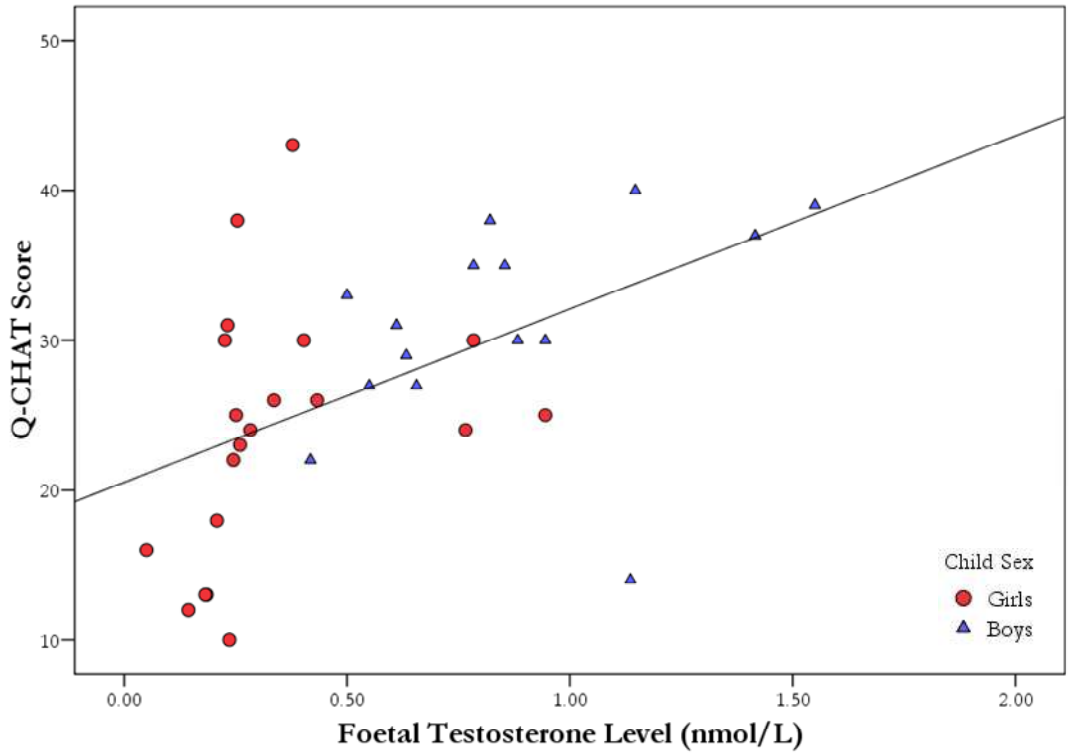
Mann-Whitney U tests showed no significant sex differences in NT levels (Mann-Whitney U=127.5, p>0.05) or FO levels (Mann-Whitney U=101.0, p>0.05). Significant sex differences were found for FT levels (Mann-Whitney U=24.0, p<0.001) and for Q-CHAT score (Mann-Whitney U=70.5, p<0.01). FT levels and sex were significantly related (Spearman's rho=0.72, p<0.001). However, no relationships between FT levels and FO levels (Spearman's rho=0.03, p>0.05) were found. In addition, no relationship was observed between NT levels and sex (Spearman's rho=-0.13, p>0.05), between FT and NT levels (Spearman's rho=0.13, p>0.05) or between NT levels and FO levels (Spearman's rho=-0.11, p>0.05).

Table 7.10. Final Regression Model for Q-CHAT scores

		Final Regression Model					
Outcome	Predictors	R^2	ΔR^2	B	SE	β	Sig
		Group					
Q-CHAT Score	Maternal age	0.05	0.05	0.07	0.30	0.04	p>0.05
	FT level	0.26	0.21	11.20	3.77	0.49	p<0.001
		Girls only					
Q-CHAT Score	Child age	0.12	0.12	0.99	1.55	0.15	p>0.05
	Older brothers			5.01	4.66	0.26	p>0.05
		Boys only					
Q-CHAT Score	Maternal age	0.00	0.00	0.11	0.53	0.05	p>0.05

For boys and girls together, the variable that met criteria for entry into the first stage of the hierarchical regression analysis was maternal age (Spearman's rho=-.32, p<0.20). FT level and Sex were tested for entry in the second stage. The FT/Sex interaction and the FT/NT interactions were tested for entry using the stepwise method in the third stage. FT level was the only variable included in the final regression model and produced a significant F-change (F-change=8.82, p<0.01, ΔR^2=0.21).

Figure 7.3. FT level and Q-CHAT scores for subset of children



Within sex analyses showed that for girls, child age (Spearman's rho=0.35, p<0.20) and older brothers (Spearman's rho=0.33, p<0.20) met entry criteria for the regression analysis. No suppressor variables were observed. FT level was tested for entry in the second stage, and the third stage tested for the interaction between FT and NT levels. The final regression model did not include FT level or the FT/NT interaction. For boys alone, maternal age (Spearmans' rho=-.39, p<0.20) was the only variable that met regression entry criteria, and FT level was tested for entry in the second stage. Results showed that FT level and the FT/NT interaction were not significant predictors of Q-CHAT scores.

7.4. Discussion

This study examined the relationships between foetal testosterone (FT) and oestradiol (FO) levels, neonatal testosterone (NT) levels and scores on the Quantitative Checklist for Autism in Toddlers (Q-CHAT) measure of autistic traits. Measurements obtained in Study 1 verified that sex differences in autistic traits are present in toddlers, with boys

scoring higher than girls. FT level was the only variable found to be significantly related to Q-CHAT scores when the sexes were combined and when girls and boys were examined separately. In addition, a significant association was found between FT levels and FO levels, but no association between Sex and FO levels ($r=-0.02$, $p>0.05$) was found. FT level was also the only predictor retained in the final regression model, whereas Sex and the FT/Sex interaction were not included, suggesting that this is an effect of FT, rather than Sex. This corresponds with previous findings in older children.

Findings from Study 2 also suggest that FT levels are positively associated with Q-CHAT scores when the boys and girls were combined. However, in this subset of children, no significant relationship was observed when girls and boys were examined separately. This may be because the within sex analyses reduced the sample size and power of the analysis. Consistent with previous findings, no relationships between FO levels and outcome were observed. In addition, no relationships were observed between FT, FO or NT levels. It is possible that this sample size was not large enough to detect differences. Nevertheless the consistent relationships observed between FT levels and Q-CHAT scores in the subset of children when the sexes were combined provide support for the notion that it is FT levels rather than NT levels that are involved in the development of autistic traits in toddlers. No sex differences were observed in NT levels and FO levels. This lack of sex differences suggests that these would not be good candidate hormones for the investigation of effects of hormone-behaviour relations.

Whilst no effect for NT was found in this study, there are limitations when using salivary measures of testosterone. For example, testosterone levels in saliva can be substantially influenced during the process of sample collection, are susceptible to interference effects caused by the leakage of blood (plasma) into saliva, and are sensitive to storage conditions when samples have been archived (Granger et al., 2004). Measurements taken from salivary samples may also be different from measurements taken from serum samples. Future research is needed to determine if neonatal serum hormone levels are related to salivary levels as well as later behaviour. In addition, it

would be interesting to further examine testosterone levels in infants in larger samples as well as in children of mothers who have not undergone amniocentesis to test whether these results are consistent in larger population samples.

In summary, results show that FT levels are positively related to Q-CHAT scores in this sample of typically developing children. Examination of NT levels showed no significant sex differences and no relationships with FT levels or with Q-CHAT scores in a subset of children. The relationship between FT levels and Q-CHAT scores remained consistent, despite the smaller sample size. Taken together, these results provide further support for the possibility that FT levels are associated with the development of autistic traits.

Chapter 8: General Discussion

This chapter provides a summary of the empirical findings and a general discussion of limitations and future directions related to this work.

8.1. Research Objectives

The Extreme Male Brain (EMB) theory proposes that autism is an exaggeration of typical sex differences in empathising and systemising ability. Although this theory originally defined the "male" and "female" brain purely in psychometric terms, it has since been suggested that increased levels of prenatal androgens (particularly testosterone) may produce excessive masculinisation of the brain and thereby increase the risk for Autism Spectrum Conditions (ASC) (Baron-Cohen et al., 2004).

It is widely accepted that hormones play an important role in sex-typical development. Studies examining behaviour in nonhuman mammals clearly show that the same prenatal hormones responsible for sexual differentiation of the body are also involved in sexual differentiation of behaviour (Breedlove, 1992; Goy & McEwen, 1980). Behavioural effects of early hormones in nonhuman primates have shown that female monkeys exposed to excess androgens early in development are masculinised with respect to sexual behaviour, rough play, grooming and some aspects of learning (Bachevalier & Hagger, 1991; Goy et al., 1988).

In humans, the presence or absence of specific hormones (and their receptors) is essential for sexual differentiation of the foetus. In addition to affecting development of physical characteristics such as genitalia (Fuchs & Klopper, 1983; Hines, 2004; Kimura, 1999; Novy & Resko, 1981; Tulchinsky & Little, 1994), there is increasing evidence that prenatal hormones have a significant effect on gender-typical aspects of behaviour (Cohen-Bendahan et al., 2005a; Hines, 2004).

The incidence of ASC is strongly biased towards males (Bryson & Smith, 1998; Fombonne, 2005; Tidmarsh & Volkmar, 2003) with a male:female ratio of 4:1 for classic autism (Chakrabarti & Fombonne, 2005) and as high as 8:1 for Asperger Syndrome (Scott et al., 2002a). The cause of the observed sex difference in ASC remains a topic of debate.

Evidence implicating prenatal testosterone in the development of autistic traits has come from studies of girls with CAH who are exposed to abnormally high levels of foetal testosterone and exhibit more autistic traits than their unaffected sisters, measured using the Autism Spectrum Quotient (AQ) (Knickmeyer et al., 2006a). Studies using putative proxy indicators of prenatal testosterone levels have also found lower (more masculinised) digit ratios in children with autism. Similar findings were reported in siblings and parents of children with autism, indicating a genetic basis for elevated FT levels in autism (Manning et al., 2001; Milne et al., 2006).

The focus of the series of studies reported in this thesis was to further examine the hypothesis that exposure to high FT levels affects performance on tasks which typically demonstrate sex differences. In addition, the possibility that FT levels are associated with traits related to ASC was also examined.

8.2. Summary of Results

Table 8.1 describes the measures used to identify sex differences in behaviour and the links with FT for boys and girls together. For each measure, Table 8.1 shows the direction of the sex differences (if present) and the effect size, calculated using Cohen's d. The final columns indicate whether the item was correlated with FT and whether FT levels (independent of sex) was a significant predictor in the regression analyses.

Table 8.1. Foetal Testosterone study results

Chapter	Characteristic	Measure	Sex Diff.	Cohen's d	Correlation with FT	FT Sig, Predictor
Ch. 2 FT and Spatial Ability	Full IQ	WASI	No	0.48	.10	--
	Block Design	WASI – Block Design Subscale	No	0.27	.19	--
	Embedded Figures	Embedded Figures Test	Yes (M>F)	0.62	.57**	Yes
	Targeting	Ball-Throwing Task	No	0.30	.11	--
	Mental Rotation	Correct items	No	0.46	.14	--
Ch. 3 FT and Gender- typical Behaviour	Gender-typical Play	PSAI Total	Yes (M>F)	2.79	.63**	Yes
		PSAI Female Sum	Yes (F>M)	1.81	-.58**	Yes
		PSAI Male Sum	Yes (M>F)	1.81	.55**	Yes
	Gender-role Behaviour	BSRI Femininity	Yes (F>M)	0.54	-.05	--
		BSRI Masculinity	Yes (M>F)	0.35	.27**	Yes
Ch. 4 FT and Aggression	Aggressive Behaviour	CBCL-A	No	0.23	.05	--
		CAS-P	No	0.25	.05	--
Ch. 5 The E-S Theory in children	Empathising	EQ-C	Yes (F>M)	0.55	-.21**	No
	Systemising	SQ-C	Yes (M>F)	0.49	.31**	Yes
	Brain Types	D	Yes (M>F)	0.73	.36**	Yes
Ch. 6 The EMB theory	Autistic traits	AQ-Child	Yes (M>F)	0.87	.41**	Yes
		CAST	Yes (M>F)	0.30	.25**	Yes
Ch. 7 Autistic traits in toddlers	Autistic traits	Q-CHAT	Yes (M>F)	0.46	.40**	Yes

Correlations reported for both sexes combined
-- denotes that a regression analysis was not conducted

Chapter 2 examined the relationship between FT levels measured in amniotic fluid and performance in a series of cognitive tasks that have shown sex differences in adults: Mental Rotation, the Embedded Figures Test and Targeting. Sex differences were confirmed in Embedded Figures Test scores, with boys scoring higher than girls. Results showed that FT was strongly correlated with Embedded Figures scores in both boys and girls. No significant sex differences or associations with FT levels were found

for IQ, Mental Rotation or Targeting. The non-significant findings in these variables are consistent with some similar findings in literature. From these results, it is unclear whether future studies of these abilities would find relations with FT levels if they also detect sex differences, unlike the current studies which have not shown sexual dimorphism in the areas of IQ, Mental Rotation or Targeting.

Chapter 3 investigated whether FT is related to childhood gender-related behaviour in n=207 typically developing children. Using two previously developed measures, clear sex differences were observed for both masculinity and femininity components of each measure. For the Pre-School Activities Inventory (PSAI), FT levels were found to predict more masculine play behaviour. For the Bem Sex Role Inventory (BSRI), FT levels significantly predicted masculinity scores, but not femininity scores. These findings suggest a role for FT in the development of gender-typical behaviour.

Chapter 4 examined the relationship between FT and scores on two measures of aggressive behaviour in a sample of n=235 children. Although several studies have identified a male tendency towards increased aggression, no sex differences were found for either measure examined here. In addition, no relationships were observed between FT levels and aggression. For this study, both measures of aggression reported skewed results with a bias towards low scores, and it is possible that the measures used did not reflect the normal range of aggressive behaviour observed in children. It may be useful for future investigations of aggressive behaviour to examine multiple measures including parent, teacher and peer report in conjunction with independent observation in order to establish the existence of any major sex differences in a more objective manner. Other studies have reported a link between aggression and current testosterone levels in both adolescence and adulthood and it is possible that the expression of aggression is more dependent on circulating rather than prenatal testosterone levels.

Chapter 5 explored the E-S theory of sex differences in a large sample of n=1256 typically developing children and n=265 children with an ASC diagnosis. Study 1

reported the development of the children's versions of the combined Empathising Quotient (EQ-C) and Systemising Quotient (SQ-C). Empathising and systemising in children showed similar patterns of sex differences to those measured in adults. The profile from children with an ASC fitted a 'hyper-masculinisation' profile, irrespective of sex.

In a second cohort of n=208 children, scores on the EQ-C and SQ-C were compared with FT levels measured prenatally. Sex differences were repeated for EQ-C and SQ-C scores. Examination of the effect of FT indicated a significant negative correlation with EQ-C score. However, FT was not retained in the final model of a regression analysis, suggesting that child sex played a larger role than FT in determining EQ-C score. A positive relationship between FT and SQ-C scores was observed. Sex and the FT/Sex interaction were not included in the regression model suggesting that FT, rather than child sex, predicts SQ-C scores.

Chapter 6 specifically investigated the relationship between prenatal exposure to testosterone and the development of autistic traits in children. Evaluation of autistic traits was assessed using the Childhood Autism Spectrum Test (CAST) and a modified version of the Autism Spectrum Quotient for children (AQ-Child). Although the CAST was specifically developed to screen for ASC in children, because of its skewed distribution it may be less useful for measuring the range of autistic traits in research studies. The AQ was developed as a measure of different characteristics of behaviour associated with ASC in a wider population of adults and adolescents, and has the advantage of being fairly normally distributed.

In Study 1, the AQ was modified for children (named AQ-Child) and administered to a general population sample (n=1225) and to a sample of children with an Autism Spectrum Condition (ASC) (n=540). Results showed a significant difference in scores between those with an ASC diagnosis and the general population. Scoring patterns in

children were similar to adults, with typical girls scoring the lowest and children with ASC scoring the highest on the AQ-Child.

In Study 2, the link between FT levels, CAST and AQ-Child score was also examined in n=235 children. Sex differences were found for both measures, with boys scoring significantly higher than girls. FT levels were positively associated with higher scores on the CAST and the AQ-Child. These results provide support for the EMB theory of autism and a role for foetal androgens in the development of male-typical behaviour and autistic traits.

Chapter 7 is an examination of the relationships between FT levels, foetal oestradiol (FO) levels and neonatal testosterone (NT) levels and their relationship to the development of autistic traits, measured using the Quantitative Checklist for Autism in Toddlers (Q-CHAT). Study 1 reported an investigation of the relationship between FT levels and autistic traits in n=129 typically developing toddlers. The Q-CHAT also revealed a significant sex difference in autistic traits, as observed for older children using the AQ-Child. In a regression analysis, the only main predictor of Q-CHAT score was FT levels, with both Sex and the FT/Sex interaction excluded from the model.

Study 2 examines the relationships between FT levels, FO levels, NT levels and autistic traits in a subset of children (n=35) from Study 1. No relationships between FO, NT levels and Q-CHAT scores were observed. However, sample size for this study was small and it would be interesting to see if similar results were reported for larger sample sizes. In addition, FO and NT levels showed no sex differences or relationships with FT levels. A relationship between FT and Q-CHAT was also observed in this subset of children whose mothers agreed to bring their child in for salivary sample collection. Sex differences were also observed in Q-CHAT scores for Study 2. These studies provide additional support for the EMB theory of autism and suggest that FT level is associated with higher Q-CHAT scores.

8.3. Within sex relationships

Levels of FT are typically much higher in boys than girls. If increased exposure to FT is sufficiently responsible for changes in sex-typical behaviour, it may be possible to observe a link between behaviour and FT level using within-sex analyses. Table 8.2 shows a summary of results within sex for variables which demonstrated a significant correlation with FT shown in Table 8.1.

Table 8.2. Within sex results and outcome variables

Chapter	Measure	FT Sig. Predictor for all cases	FT Sig. Predictor for Girls	FT Sig. Predictor for Boys	Variables included in stepwise stage of regression
Chapter 2	Embedded Figures Test	Yes ΔR^2=.40	Yes ΔR^2=.68	Yes ΔR^2=.18	FT level FT X Sex interaction
Chapter 3	PSAI Total	Yes ΔR^2=.03	Yes ΔR^2=.20	No	FT level FT X Sex interaction
	PSAI Female Sum	Yes ΔR^2=.03	Yes ΔR^2=.07	No	Sex FT level FT X Sex interaction
	PSAI Male Sum	Yes ΔR^2=.03	Yes ΔR^2=.07	No	Sex FT level FT X Sex interaction
	BSRI Masculinity	Yes ΔR^2=.07	Yes ΔR^2=.09	No	FT level
Chapter 5	EQ-C	No	No	No	Sex
	SQ-C	Yes ΔR^2=.10	Yes ΔR^2=.23	No	FT level
	D	Yes ΔR^2=.12	Yes ΔR^2=.22	No	FT level Sex FT X Sex interaction
Chapter 6	AQ-Child	Yes ΔR^2=.16	Yes ΔR^2=.08	Yes ΔR^2=.05	FT level Sex
	CAST	Yes ΔR^2=.05	No	Yes ΔR^2=.06	FT level
Chapter 7	Q-CHAT	Yes ΔR^2=.16	Yes ΔR^2=.10	Yes ΔR^2=.13	FT level

Note: Only outcome variables with significant FT correlations are shown.
Variables included in regression analysis are reported for both sexes combined

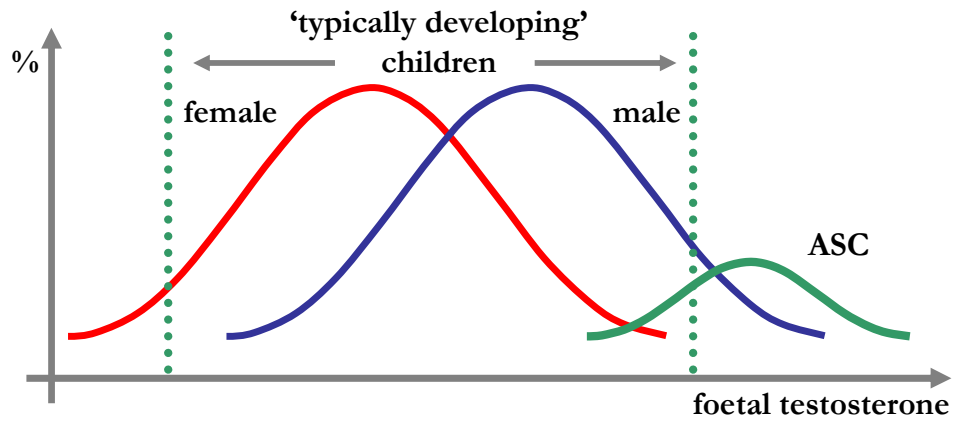
Table 8.2 shows that within sex correlations with FT levels were seen for most of the variables that show a sex difference. The Table also shows that within sex effects of FT were observed more often in girls than boys. Other studies have also reported

relationships between FT and behaviour in girls and not boys. The current findings are consistent with previous studies in samples such as CAH, which have generally found stronger correlations between hormone levels and male-typical behaviour in girls rather than boys (Berenbaum, 1999; Berenbaum & Hines, 1992; Berenbaum & Snyder, 1995; Ehrhardt & Baker, 1974; Hines et al., 2004). An exception to this was found in CAST scores which demonstrated a correlation in boys only.

Several reasons might account for the bias of within sex correlations towards girls. Females might be particularly sensitive to changes in FT level or androgen may need to be very high before sex-typed activity preference is masculinised in boys. It has also been proposed that the effect of elevated FT exposure may produce increasing masculinisation up to a certain dose, but additional exposure may cause a reversal toward the original state (demasculinisation) (Knickmeyer et al., 2007).

A common feature of all the studies presented in Table 8.2 is that they focus on typically developing children. Children with ASC have not been included in the analyses, since a much larger sample in which to measure foetal hormone levels would be needed. A further possible explanation for the lack of correlation between behaviours and FT levels in boys is that 'male extreme' hormone levels may result in ASC and are excluded by default, since only typically developing children were included in these studies (see Figure 8.1). In contrast, 'female extremes' of FT level simply result in 'masculinised' female behaviour and are included in the samples. Exclusion of boys with very high FT levels might be expected to reduce within sex differences in boys.

Figure 8.1. Hypothetical foetal testosterone levels in typical and ASC populations



For CAST scores (see Table 8.2), the presence of a within sex difference in boys only might be due to the skewed responses for boys and girls. In fact FT is the only predictor of CAST score, further suggesting that within sex relationships for boys are only found for extreme cases. Other measures specifically examining autistic traits (AQ-Child and Q-CHAT) identify correlations between FT level and autistic traits within both sexes.

It is noteworthy that the ΔR^2 values were similar between boys and girls for the AQ-Child and Q-CHAT, suggesting that the effect of FT is similar for both sexes for these measures. These are both measures of autistic traits that have more normal distributions than the CAST. Perhaps the relatively large and more normal variability in scores lends power to detect relationships between FT levels and scores. It would be important for future research to replicate these findings in larger, general population studies using diagnostic measures of autism such as the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000; Lord, Rutter & Le Couteur, 1994).

The only measure which does not report within sex differences for either sex is EQ-C score. Inspection of results from the EQ-C suggests that there is a significant correlation with FT when the sexes were combined. However, FT levels were not retained in the final model of the regression analysis and showed no significant

correlations within sex. Perhaps this is because FT is not the dominant effect on empathising ability measured using the EQ-C. This is consistent with the suggestion that FT levels do not account for all differences in sex-typical behaviour or the onset of autistic traits and that a variety of factors are likely to be at work.

The Embedded Figures Test (EFT) showed the largest effect of FT, but not the largest sex differences (d=0.57). Results for the EFT showed that when the sexes were combined, 40% of the variance is predicted by FT levels. In girls, 68% of the scores were predicted by FT levels and in boys it was 18%. In contrast, the measure which showed the largest sex difference was the Pre-School Activities Inventory (PSAI) which had an effect size of d=2.79. For the PSAI, no correlation with FT was observed in boys, whereas for girls 20% of the variability in scores was predicted by FT. It appears that the FT-outcome relationship is not dependent on the magnitude of sex differences, further suggesting that the development of sex differences in behaviours are dependent on a variety of influences.

8.4. Additional considerations

The relationships between prenatal hormones and behaviours that show sex differences in humans are likely to be dependent on many factors and these studies only report correlations with hormone levels measured at a single time point. Research in animals has generally shown that hormonal effects on sexually dimorphic behaviour may be dose and time-dependent, with increased masculinisation occurring for higher levels of androgen (Cohen-Bendahan et al., 2005a; Hines, 2004). The present results in humans are generally in line with findings in individuals with CAH showing that FT masculinises behaviour in domains that show sex differences in girls. The current results also tend to suggest that using measures sensitive to 'extreme' forms of male behaviour, elevated FT exposure may also be related to masculinisation in girls and perhaps 'hyper-masculinisation' in boys.

The effects of FT may also be non-linear. Previous studies have shown non-linear relationships with prenatal hormones and behaviour in humans (Lutchmaya et al., 2002a). Research in nonhumans also suggests that the hormone levels required to affect development also differ across behaviours (Goy & McEwen, 1980). Moreover, the presence of one hormone may also promote or prevent the effects of another (Cohen-Bendahan et al., 2005a; Goy & McEwen, 1980). Finally, the effect of hormones is also dependent on the availability of receptors, as seen when examining individuals with Complete or Partial Androgen Insensitivity Syndrome.

The influence of all these hormonal factors on later behavioural development was outside the scope of these studies. These differing relationships in boys versus girls as well as the differing amounts of variability that FT accounts for (as shown in Tables 8.1 and 8.2), bring to attention the many factors that must be considered when exploring hormone-behaviour relations in humans.

8.5. Factors that influence FT levels

A number of studies have examined factors such as stress, which influence testosterone levels both pre and postnatally. Prenatal stress in male rats has been found to demasculinise and feminise adult sexual behaviour (Ward, 1977). Testosterone levels in newborn male rats are also observed to be reduced in stressed compared to non-stressed controls. There are also potentially consistent findings in humans, such as homosexual men reporting more maternal stressors (such as bereavement) during pregnancy, relative to controls (Dorner et al., 1983). In females, there is some evidence that prenatal stress is associated with masculinised gender role and sexual behaviour (Hines et al., 2002b).

A recent study by Gitau et al. (2005) has shown that FT levels measured from foetal plasma samples correlated positively with both foetal cortisol (assumed to be a reflection of stress levels) and maternal testosterone concentrations in n=44 human

foetuses. Maternal plasma testosterone and foetal plasma cortisol were independently correlated with foetal plasma testosterone in both sexes. Unlike the norm in the adult, where testosterone production is often inhibited by cortisol, in the foetus a positive association has been observed (Gitau et al., 2005). One small scale study (n=12) showed that children with autism showed a more variable circadian rhythm as well as significant elevations in cortisol (indicating increased stress) following exposure to a novel, non-social stimulus compared to typical children (Corbett et al., 2006). It is difficult to control for emotional stress and further studies could also control for cortisol levels when examining the effects of hormones such as testosterone on later behaviour. Additionally, future research examining if relationships exist between serum FT and cortisol levels or amniotic FT and cortisol levels would be useful. It would also be interesting to investigate the effects of prenatal levels of cortisol to later behaviour and in children with ASC. However, this would require a longitudinal strategy in large sample sizes.

Hormone levels during pregnancy may also be influenced by the timing of previous maternal birth history. A study of umbilical cord blood has shown that the first-born children of both sexes have higher levels of oestrogen, progesterone and testosterone (Maccoby et al., 1979). This finding was independent of maternal age, length of labour or birth weight. When childbirths are spaced closely together (within 4 years), results show that hormone levels are lower than normal. After 4 years, levels return to first-born levels or above. The effects of sibling spacing has also been observed to be greater for boys than girls (Maccoby et al., 1979). It is unclear how such factors might affect results in this study, or whether these differences are replicated in foetal testosterone levels.

Genetic sex is determined at conception. However, it is accepted that genetic variation has an important role in the development of ASC (Folstein & Rosen-Sheidley, 2001; Gupta & State, 2007; Lauritsen & Ewald, 2001). This is clear from the high degree of heritability observed in autism (Bailey et al., 1995; Ritvo et al., 1985). The degree to

which genetic variation is coupled with changes in hormone exposure is also unknown and it may be that changes in hormone levels are simply a manifestation of a genetic influence. This would be an interesting area for future research, since investigations of current testosterone levels have shown rates of heritability between 50% and 66% (Harris, Vernon & Boomsma, 1998; Hoekstra, Bartels & Boomsma, 2006).

8.6. FT and the brain

Results from the current studies suggest that higher prenatal hormone levels could be responsible for greater masculinisation of behaviour. However, it is clear that FT, measured during the second trimester of pregnancy is not the only factor contributing to the behaviours examined. We know this because it only accounts for a proportion of the variance (summarised in Table 8.2 between 0-67%, depending on which behaviour and on which sex).

Other evidence supporting the role for FT in human development comes from physical studies of brain structure. If hormones are a risk factor for ASC, then one could hypothesise that there may be an overlap between areas of the human brain that show sexual dimorphism because of their density of androgen receptors and areas of the brain that are atypical in individuals with ASC. Table 8.3 shows Knickmeyer and Baron-Cohen's (2006) comparison of brain regions that are sexually dimorphic, those that are atypical in autism and those that contain the most androgen receptors. Whilst this points at some overlap (especially in the amygdala, corpus callosum, temporal and frontal cortex), it is important to recognise that this approach does not prove that sexual dimorphism is due to the density of androgen receptors, or that the abnormalities in ASC are necessarily associated with androgen receptors. Such overlap could be purely coincidental and will require direct testing of any causal factors.

Table 8.3. Comparison of brain regions implicated in autism with those showing gross anatomical sex differences and those expressing androgen receptors

Autism	Androgen Receptors	Sexually Dimorphic (Gross anatomical level)
Parietal-temporal lobe	Temporal lobe	Parietal and Temporal lobe
Cerebellum	Cerebellum	
Amygdala	Amygdala	Amygdala
Hippocampus		
Corpus callosum	Corpus callosum	Corpus callosum
Frontal cortex	Frontal cortex	Frontal cortex
	Hypothalamus	Hypothalamus
	Cingulate Cortex	

Table from: Knickmeyer, R. C., & Baron-Cohen, S. (2006). Fetal testosterone and sex differences in typical social development and in autism. *Journal of Child Neurology*, *21*, 825-845.

8.7. Limitations

Human behaviour is complex and biological, social or cultural factors are continuously interacting, making it challenging to investigate the causes of behaviour. In this thesis there has been no attempt to review or test the role of genetics in sexual differentiation of behaviour or in the development of autistic traits or ASC, but these areas are reviewed elsewhere (Collaer & Hines, 1995; Folstein & Rosen-Sheidley, 2001; Goy & McEwen, 1980; Gupta & State, 2007; Hines, 2004; Kimura, 1999; Lauritsen & Ewald, 2001). To the extent that social factors have been considered within the current series of experiments, these have been restricted to certain demographic variables (such as maternal age, parental education, and number of siblings), and it is acknowledged that behaviours such as 'systemising', 'empathising' or gender-typical play are likely to be influenced by a range of social factors that have not been measured in these studies.

8.7.1. *Limitations of amniocentesis*

As mentioned in Chapter 1, direct studies of the effects of prenatal testosterone are difficult because levels rise and fall in the foetal environment over the course of gestation. The optimal way to directly measure prenatal testosterone exposure is

currently via amniotic fluid obtained during clinical amniocentesis for the ethical reasons outlined below. However, this method is not ideal because it limits the sample available. Typically only single measures are available, since amniocentesis is not repeated. In addition, it does not allow exploration of the effect of varying hormone levels or response to sensitive periods for development. To test for any variation in the timing of amniocentesis (since the window in which this is typically performed ranges from 12 to 19 weeks gestation), we entered gestational age as a factor into all analyses. Surprisingly, gestational age was not a significant predictor of FT levels.

A drawback of amniocentesis is that it can only be conducted for purposes of diagnosing foetal anomalies. This means that the samples studied are selected in several ways that may influence the generalisability of results. A random sample of pregnancies undergoing amniocentesis would not be ethical to collect because of the risks involved in the procedure: approximately 1% of amniocenteses result in miscarriage (d'Ercole et al., 2003; Sangalli, Langdana & Thurlow, 2004). In addition, in these amniotic fluid studies, total extractable (or free) testosterone is utilised. However, free testosterone may not be directly related to exposure to the androgen receptors in the brain (Hines, 2004). Finally, amniocentesis is typically performed on women who are older (age 35 years and above) which may confound any results obtained. To guard against this, we controlled for maternal age in the studies reported in this thesis.

8.7.2. *Limitations in the psychological measures used*

The studies presented here rely heavily on maternal report and mothers may interpret individual questionnaire items differently. Ideally, correlations established in these experiments would be repeated in vivo, which would allow for less subjective results. However, an advantage of maternal report is that mothers observe their children's strengths and weaknesses in a variety of contexts and over an extended period of time. Parental report also allows for research with a much larger sample size than in vivo

testing and/or naturalistic observation. In addition, mothers participating in the Cambridge FT Project are obviously blind to their children's FT levels.

8.7.3. *Limitations in generalisability of results*

Results from these studies suggest that variations in FT levels are related to aspects of sexually dimorphic behaviour and cognition in typically developing children. However, extrapolating these results to individuals with a formal diagnosis of ASC needs to be done with caution. The sample sizes of the current FT studies are too small to be able to test if FT levels are elevated in formally diagnosed cases of ASC, since these have a prevalence rate of about 1% (Baird et al., 2006), and a sample size of thousands would be required. A large-scale collaboration is currently underway so as to increase sample sizes sufficiently to compare FT levels in cases of ASC versus controls.

Against these limitations, the strength of the amniocentesis design is that it provides a quantifiable measure of foetal exposure to testosterone from the prenatal environment, whilst avoiding unnecessary additional risk associated with serum sample collection during a period in which it is hypothesised that masculinisation of the brain occurs. Some previous studies investigating the relationship between FT and cognitive development in humans have relied on individuals with abnormal hormonal environments during pregnancy, such as those with CAH, or those exposed to drugs that mimic or block natural hormones (Hines et al., 2003b; Knickmeyer et al., 2006a; Pasterski et al., 2005; Servin et al., 2003). In these cases it is difficult to differentiate between the effects of the hormonal environment, a genetic abnormality associated with the disorder, or any additional effects that drugs may produce. It is probable that the current sample is more representative of the general population than studies based on abnormal environments.

8.8. Future Directions

There is converging evidence supporting a role for prenatal hormones in the development of sexually dimorphic behaviour. It is possible that genetic influences are responsible for or interact with prenatal hormone levels which lead to the development of ASC. Considering the current support for a role for FT in the development of autistic traits, it would be beneficial for future studies to examine the relationships between FT levels, genetic variation and the development of autistic traits.

Although measures of IQ, Mental Rotation, Targeting and aggression used in this study did not show sex differences, other measures have reported significant differences between males and females in these behaviours. It would be useful for future studies to explore relations with FT levels if they do detect sex differences.

The replication of the current results in larger sample sizes would also help to increase the range of FT levels observed in these studies and assist in identifying any factors that are linked with levels in the extreme ranges. Future studies could assess whether relationships between FT levels and the development of autistic traits are consistent for individuals with a clinical diagnosis of ASC, since the current samples only included typically developing children.

The study of empathising and systemising in children indicated that although FT was a significant predictor of SQ-C score, this was not the case for EQ-C scores. Future studies could examine whether the relationships between FT levels remain consistent using other measures of empathy (e.g. observational, experimental, etc.). It has been suggested that genetic factors may influence EQ score in adults, and these might be related to sex hormones. Future research could therefore explore if genes are related to the expression of empathy.

It would also be valuable to further establish the relationships between direct measures of hormones (e.g. amniotic fluid or serum measures) and physical characteristics (e.g. 2D:4D ratio or dermatoglyphics) which have been used as proxy measures of hormone exposure. The benefit of using these types of measurements is that they are easy to obtain and have also been linked to multiple areas of human development. However, limited evidence exists for a relationship between these proxy measures and exposure to prenatal hormones. If such a link was confirmed using direct measures of hormones, it could simplify future investigations of hormone effects.

The EMB theory of autism has been developed from studies in typically developing and high-functioning individuals. It would be interesting to extend the scope of this theory with an examination of individuals with more severe forms of ASC. It is also difficult to assess the validity of 'empathising' and 'systemising' measures and future studies could further explore how these domains develop and also how they are measured. In addition, it would be important to examine how these abilities are expressed in the brain.

8.9. Conclusions

Autism Spectrum Conditions (ASC) are characterised by social impairments, restricted and repetitive interests accompanied by language delay. ASC are believed to lie on a spectrum, reflecting the range of individual ability in each of these areas. Some of the behaviours which are characteristic of ASC have been linked to extremes of certain male-typical behaviours. Evidence includes superior performance on a range of tasks where male individuals typically outperform females and impairment on tasks with female superiority.

Research suggests that gender-typical behaviours may be affected by gonadal hormones, in particular foetal exposure to testosterone. The objective of the current studies was to examine the link between FT levels (measured in amniotic fluid) and a series of sexually

dimorphic behaviours. Results suggest that prenatal exposure to elevated FT levels enhances masculinisation of certain behaviours. In addition, direct measurement of autistic traits was used to examine whether these measures of behaviour are consistent with the EMB theory of autism. It was striking that on all three measures (CAST, AQ-Child, and Q-CHAT), FT positively predicted number of autistic traits.

Not all of the measures used in these studies found the anticipated sex differences. Where sex differences were found, there tended to also be a correlation with FT levels. For some measures of typical behaviour, FT was also associated with more male-typical behaviour. Within sex analyses suggest that the relationships with FT were stronger in girls, who are generally exposed to lower testosterone levels in typical development. These findings are consistent with a role for FT in the development of cognitive sex differences and autistic traits.

Experimental analysis of empathising and systemising in children revealed similar patterns to those found in adults, providing further support for the E-S theory of sex differences. Further examination revealed that whilst there was a significant negative correlation between empathising (EQ-C) score and FT levels, FT levels were not retained in the final regression model as a significant predictor. FT was, however, the only predictor of systemising (SQ-C) scores in children.

Although measurements report some inconsistencies and differing correlation strength, results suggest an overall tendency for increased male-typical behaviour with higher FT levels. Measurements which report behaviours linked to autism are generally consistent with the Extreme Male Brain theory. Finally, results suggest a role for exposure to high levels of FT in the development of autistic traits.

In summary, FT levels have been found to be significantly related to some, but not all, male-typical traits, and lend further support for a role of FT levels in the development of behaviours related to sex differences. The findings presented also lend support to the

EMB theory of ASC and to a further relationship with foetal testosterone levels. Although higher levels of FT are unlikely to be the sole the cause of autism, the studies reported here provide evidence for a role of FT in the development of autistic traits in typically developing children. This remains to be tested in clinical samples. It is hoped that results from this series of studies may enable further understanding of the aetiology of ASC and of typical variation in sexually dimorphic behaviour.

References

Abramovich, D. R., & Rowe, P. (1973). Foetal plasma testosterone levels at mid-pregnancy and at term: Relationship to foetal sex. *Journal of Endocrinology*, *56*, 621-622.

Achenbach, T., & Edelbrock, C. (1983). *Manual for the Child Behavior Checklist*. Burlington: University of Vermont.

Alexander, G. M., & Hines, M. (1994). Gender labels and play styles: Their relative contribution to children's selection of playmates. *Child Development*, *65*, 869-879.

Alexander, G. M., & Hines, M. (2002). Sex differences in response to children's toys in nonhuman primates (cercopithecus aethiops sabaeus). *Evolution and Human Behavior*, *23*, 467-479.

Allen, M. H., Lincoln, A. J., & Kaufman, A. S. (1991). Sequential and simultaneous processing abilities of high-functioning autistic and language-impaired children. *Journal of Autism and Developmental Disorders*, *21*, 483-502.

Allison, C., Baron-Cohen, S., Wheelwright, S., Charman, T., Richler, J., Pasco, G., & Brayne, C. (2008). The Q-CHAT (Quantitative Checklist for Autism in Toddlers): A normally distributed quantitative measure of autistic traits at 18-24 months of age: Preliminary report. *Journal of Autism and Developmental Disorders*, *Epub ahead of print*.

Allison, C., Williams, J., Scott, F., Stott, C., Bolton, P., Baron-Cohen, S., & Brayne, C. (2007). The Childhood Asperger Syndrome Test (CAST): Test-retest reliability in a high scoring sample. *Autism*, *11*, 173-185.

Altman, D. G. (1991). *Practical Statistics for Medical Research*. London: Chapman and Hall.

APA. (1994). *DSM-IV Diagnostic and Statistical Manual of Mental Disorders, 4th Edition*. Washington DC: American Psychiatric Association.

Archer, J. (1991). The influence of testosterone on human aggression. *British Journal of Psychology*, *82*, 1-28.

Archer, J. (2004). Sex differences in aggression in real-world settings: A meta-analytic review. *Review of General Psychology*, *8*, 291-322.

Austin, E. J. (2005). Personality correlates of the broader autism phenotype as assessed by the Autism Spectrum Quotient (AQ). *Personality and Individual Differences*, *38*, 451-460.

Austin, E. J., Manning, J. T., McInroy, K., & Mathews, E. (2002). A preliminary investigation of the associations between personality, cognitive ability and digit ratio. *Personality and Individual Differences*, *33*, 1115-1124.

Bachevalier, J., & Hagger, C. (1991). Sex differences in the development of learning abilities in primates. *Psychoneuroendocrinology*, *16*, 177-188.

Bailey, A., Le Couteur, A., Gottesman, I., Bolton, P., Simonoff, E., Yuzda, E., & Rutter, M. (1995). Autism as a strongly genetic disorder: Evidence from a British twin study. *Psychological Medicine*, *25*, 63-77.

Bailey, A. A., & Hurd, P. L. (2005). Finger length ratio (2D:4D) correlates with physical aggression in men but not in women. *Biological Psychology*, *68*, 215-222.

Baird, G., Charman, T., Baron-Cohen, S., Cox, A., Swettenham, J., Wheelwright, S., & Drew, A. (2000). A screening instrument for autism at 18 months of age: A 6-year follow-up study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *39*, 694-702.

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., & Charman, T. (2006). Prevalence of disorders of the autism spectrum in a population cohort

of children in South Thames: the Special Needs and Autism Project (SNAP). *Lancet*, *368*, 210-215.

Barnet, V., & Lewis, T. (1978). *Outliers in statistical data*. New York: Wiley.

Baron-Cohen, S. (1987). Autism and symbolic play. *British Journal of Developmental Psychology*, *5*, 139-148.

Baron-Cohen, S. (1995). Mindblindness: An essay on autism and theory of mind. *Learning, development, and conceptual change. Cambridge, MA, US: The MIT Press*, *171*.

Baron-Cohen, S. (1999). The extreme male-brain theory of autism. In H. Tager Flusberg (Ed.), *Neurodevelopmental Disorders*. Cambridge, Massachusetts: MIT Press.

Baron-Cohen, S. (2002). The extreme male brain theory of autism. *Trends in Cognitive Sciences*, *6*, 248-254.

Baron-Cohen, S. (2003). *The Essential Difference: Men, women and the extreme male brain*. Penguin: London.

Baron-Cohen, S., Allen, J., & Gillberg, C. (1992). Can autism be detected at 18 months? The needle, the haystack, and the CHAT. *British Journal of Psychiatry*, *161*, 839-843.

Baron-Cohen, S., Cox, A., Baird, G., Sweettenham, J., & Nighingale, N. (1996). Psychological markers in the detection of autism in infancy in a large population. *British Journal of Psychiatry*, *168*, 158-163.

Baron-Cohen, S., & Hammer, J. (1997). Is autism an extreme form of the "male brain"? *Advances in Infancy Research*, *11*, 193-217.

Baron-Cohen, S., Hoekstra, R., Knickmeyer, R., & Wheelwright, S. (2006a). The Autism-Spectrum Quotient (AQ) - Adolescent version. *Journal of Autism and Developmental Disorders*, *36*, 343-350.

Baron-Cohen, S., Jolliffe, T., Mortimore, C., & Robertson, M. (1997). Another advanced test of theory of mind: Evidence from very high functioning adults with autism or Asperger Syndrome. *Journal of Child Psychology and Psychiatry*, *38*, 813-822.

Baron-Cohen, S., Knickmeyer, R., & Belmonte, M. K. (2005a). Sex differences in the brain: Implications for explaining autism. *Science*, *310*, 819-823.

Baron-Cohen, S., Lutchmaya, S., & Knickmeyer, R. (2004). *Prenatal testosterone in mind*. Cambridge, Massachusetts: The MIT Press.

Baron-Cohen, S., Richler, J., Bisarya, D., Gurunathan, N., & Wheelwright, S. (2003). The Systemising Quotient (SQ): An investigation of adults with Asperger syndrome or high functioning autism and normal sex differences. *Philosophical Transactions of the Royal Society*, *358*, 361-374.

Baron-Cohen, S., Ring, H., Chitnis, X., Wheelwright, S., Gregory, L., Williams, S., Brammer, M., & Bullmore, E. (2006b). fMRI of parents of children with Asperger Syndrome: A pilot study. *Brain Cognition*, *61*, 122-130.

Baron-Cohen, S., Scott, F. J., Allison, C., Williams, J., Bolton, P., Matthews, F. E., & Brayne, C. (in preparation). Estimating autism spectrum prevalence in the population: A school based study from the UK.

Baron-Cohen, S., Scott, F. J., Allison, C., Williams, J., Bolton, P., Matthews, F. E., & Brayne, C. (submitted). Estimating autism spectrum prevalence in the population: A school based study from the UK.

Baron-Cohen, S., & Wheelwright, S. (2003). The Friendship Questionnaire: An investigation of adults with Asperger syndrome or high-functioning autism, and normal sex differences. *Journal of Autism and Developmental Disorders*, *33*, 509-517.

Baron-Cohen, S., & Wheelwright, S. (2004). The Empathy Quotient: An investigation of adults with Asperger syndrome or high functioning autism, and normal sex differences. *Journal of Autism and Developmental Disorders*, *34*, 163-175.

Baron-Cohen, S., Wheelwright, S., Robinson, J., & Woodbury-Smith, M. (2005b). The Adult Asperger Assessment (AAA): A diagnostic method. *Journal of Autism and Developmental Disorders*, *35*, 807-819.

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E. (2001a). The Autism Spectrum Quotient (AQ) : Evidence from Asperger syndrome/high functioning autism, males and females, scientists and mathematicians. *Journal of Autism and Developmental Disorders*, *31*, 5-17.

Baron-Cohen, S., Wheelwright, S., Spong, A., Scahill, L., & Lawson, J. (2001b). Are intuitive physics and intuitive psychology independent? A test with children with Asperger syndrome. *Journal of Developmental and Learning Disorders*, *5*, 47-78.

Bates, J. E., & Bentler, P. M. (1973). Play activities of normal and effeminate boys. *Developmental Psychology*, *9*, 20-27.

Beatty, W. W., & Tröster, A. I. (1987). Gender differences in geographical knowledge. *Sex Roles*, *16*, 565-590.

Bem, S. L. (1974). The measurement of psychological androgyny. *Journal of Consulting and Clinical Psychology*, *42*, 155-162.

Berenbaum, S. A. (1999). Effects of early androgens on sex-typed activities and interests in adolescents with congenital adrenal hyperplasia. *Hormones and Behavior*, *35*, 102-110.

Berenbaum, S. A., & Hines, M. (1992). Early androgens are related to childhood sex-typed toy preferences. *Psychological Science*, *3*, 203-206.

Berenbaum, S. A., & Resnick, S. M. (1997). Early androgen effects on aggression in children and adults with congenital adrenal hyperplasia. *Psychoneuroendocrinology*, *22*, 505-515.

Berenbaum, S. A., & Snyder, E. (1995). Early hormonal influences on childhood sex-typed activity and playmate preferences: Implications for the development of sexual orientation. *Developmental Psychology*, *31*, 31-42.

Berlin, D. F., & Languis, M. L. (1981). Hemispheric correlates of the Rod-and-frame Test. *Perceptual and Motor Skills*, *52*, 35-41.

Betancur, C., Leboyer, M., & Gillberg, C. (2002). Increased rate of twins among affected sibling pairs with autism. *American Journal of Human Genetics*, *70*, 1381-1383.

Billington, J., Baron- Cohen, S., & Wheelwright, S. (2007). Cognitive style predicts entry into physical sciences and humanities: Questionnaire and performance tests of empathy and systemizing. *Learning and Individual Differences*, *17*, 260-268.

Bishop, D. V. M. (1998). Development of the children's communication checklist (CCC): A method for assessing qualitative aspects of communicative impairment in children. *Journal of Child Psychology and Psychiatry*, *6*, 879-891.

Bishop, D. V. M., Maybery, M., Maley, A., Wong, D., Hill, W., & Hallmayer, J. (2004). Using self-report to identify the broad phenotype in parents of children with autistic spectrum disorders: A study using the Autism-Spectrum Quotient. *Journal of Child Psychology and Psychiatry*, *45*, 1431-1436.

Bolton, P., Macdonald, H., Pickles, A., Rios, P., Goode, S., Crowson, M., Bailey, A., & Rutter, M. (1994). A case-control family history study of autism. *Journal of Child Psychology and Psychiatry*, *35*, 877-900.

Book, A. S., Starzyk, K. B., & Quinsey, V. L. (2001). The relationship between testosterone and aggression: A meta-analysis. *Aggression and Violent Behavior*, *6*, 579-599.

Bosacki, S., & Astington, J. W. (1999). Theory of mind in preadolescence: Relations between social understanding and social competence. *Social Development*, *8*, 237-255.

Breedlove, S. M. (1992). Sexual dimorphism in the vertebrate nervous system. *The Journal of Neuroscience*, *12*, 4133-4142.

Brown, G. R., Nevison, C. M., Fraser, H. M., & Dixson, A. F. (1999). Manipulation of postnatal testosterone levels affect phallic and clitoral development in infant rhesus monkeys. *International Journal of Andrology*, *22*, 119-128.

Brown, W. M., Hines, M., Fane, B., & Breedlove, S. M. (2002). Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Hormones and Behavior*, *42*, 380-386.

Bryson, S. E., & Smith, I. M. (1998). Epidemiology of autism: Prevalence, associated characteristics, and implications for research and service delivery. *Mental Retardation and Developmental Disabilities Research Reviews. Special Issue: Autism*, *4*, 97-103.

Buss, A. H., & Perry, M. (1992). The aggression questionnaire. *Journal of Personality and Social Psychology*, *63*, 452-459.

Cappa, S. F., Guariglia, C., Papagno, C., Pizzamiglio, L., Vallar, G., Zoccolotti, P., Ambrosi, B., & Santiemma, V. (1988). Patterns of lateralization and peformance levels for verbal and spatial tasks in congenital androgen deficiency. *Behavioural Brain Research*, *31*, 177-183.

Chakrabarti, B., & Baron-Cohen, S. (2006). Empathizing: Neurocognitive developmental mechanisms and individual differences. *Progress in Brain Research*, *156*, 403-417.

Chakrabarti, B., Hill-Cawthorne, G., Dudbridge, F., Wheelwright, S., Allison, C., & Baron-Cohen, S. (submitted). Candidate genes associated with Asperger syndrome and four measures of autistic traits and the 'broader autism phenotype'.

Chakrabarti, S., & Fombonne, E. (2005). Pervasive developmental disorders in preschool children: Confirmation of high prevalence. *American Journal of Psychiatry*, *162*, 1133-1141.

Clark, M. M., & Galef, B. G. (1998). Effects of intraurine position on the behavior and genital morphology of litter-bearing rodents. *Developmental Neurology*, *14*, 197-211.

Cohen-Bendahan, C. C., van de Beek, C., & Berenbaum, S. A. (2005a). Prenatal sex hormone effects on child and adult sex-typed behavior: Methods and findings. *Neuroscience and Biobehavioral Reviews*, *29*, 353-384.

Cohen-Bendahan, C. C. C., Buitelaar, J. K., van Goozen, S. H. M., Orlebeke, J. F., & Cohen-Kettenis, P. T. (2005b). Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls. *Hormones and Behavior*, 230-237.

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences (2nd ed.)*. Hillsdale:New Jersey: Lawrence Erlbaum Associates.

Cole-Harding, S., Morstad, A. L., & Wilson, J. R. (1988). Spatial ability in members of opposite-sex twin pairs (abstract). *Behavior Genetics*, *18*, 710.

Collaer, M. L., & Hines, M. (1995). Human behavioural sex differences: A role for gonadal hormones during early development? *Psychological Bulletin*, *118*, 55-107.

Connellan, J., Baron-Cohen, S., Wheelwright, S., Batki, A., & Ahluwalia, J. (2000). Sex differences in human neonatal social perception. *Infant Behavior and Development*, *23*, 113-118.

Constantino, J. N., Gruber, C. P., Davis, S., Hayes, S., Passanante, N., & Przybeck, T. (2004). The factor structure of autistic traits. *Journal of Child Psychology and Psychiatry*, *45*, 719-726.

Constantino, J. N., Przybeck, T., Friesen, D., & Todd, R. D. (2000). Reciprocal social behaviour in children with and without pervasive developmental disorders. *Developmental and Behavioural Pediatrics*, *1*, 2-11.

Constantino, J. N., & Todd, R. D. (2003). Autistic traits in the general population. *Archives of General Psychiatry*, *60*, 524-530.

Corbett, B. A., Mendoza, S., Abdullah, M., Wegelin, J. A., & Levine, S. (2006). Cortisol circadian rhythms and response to stress in children with autism. *Psychoneuroendocrinology*, *31*, 59-68.

Coyne, S. M., Manning, J. T., Ringer, L., & Bailey, L. (2007). Directional asymmetry (right-left differences) in digit ratio (2D:4D) predict indirect aggression in women. *Personality and Individual Differences*, *43*, 865-872.

Croen, L. A., Grether, J. K., & Selvin, S. (2002). Descriptive epidemiology of autism in a California population: Who is at risk? *Journal of Autism and Developmental Disorders*, *32*, 217-224.

Csatho, A., Osvath, A., Bicsak, E., Karadi, K., Manning, J., & Kallai, J. (2003). Sex role identity related to the ratio of second to fourth digit length in women. *Biological Psychology*, *62*, 147-156.

Cutting, A. L., & Dunn, J. (1999). Theory of mind, emotion understanding, language, and family background: Individual differences and interrelations. *Child Development*, *70*, 853-865.

d'Ercole, C., Shojai, R., Desbriere, R., Chau, C., Bretelle, F., Piechon, L., & Boubli, L. (2003). Prenatal screening: Invasive diagnostic approaches. *Child's Nervous System*, *19*, 444-447.

Davison, K. K., & Susman, E. J. (2001). Are hormone levels and cognitive ability related during early adolescence? *International Journal of Behavioral Development*, *25*, 416-428.

Dawood, M. Y., & Saxena, B. B. (1977). Testosterone and dihydrotestosterone in maternal and cord blood and in amniotic fluid. *American Journal of Obstetrics and Gynecology*, *129*, 37-42.

De Giacomo, A., & Fombonne, E. (1998). Parental recognition of developmental abnormalities in autism. *European Child and Adolescent Psychiatry*, *7*, 131-136.

De Vries, G. J., & Simerly, R. B. (2002). Anatomy, development, and function of sexually dimorphic neural circuits in the mammalian brain. In D. W. Pfaff, A. E. Arnold, A. M. Etgen, S. E. Fahrbach & R. T. Rubin (Eds.), *Hormones, Brain and Behavior* (Vol. 4, pp. 137-191). San Diego, CA: Academic Press.

Dorner, G., Schenk, B., Schmiedel, B., & Ahrens, L. (1983). Stressful events in prenatal life of bi- and homosexual men. *Experimental Clinical Endocrinology*, *81*, 83-87.

Ehrhardt, A. A., & Baker, S. W. (1974). Fetal androgens, human central nervous system differentiation, and behavior sex differences. In R. C. Friedman, R. R. Richart & R. L. Vande Wiele (Eds.), *Sex differences in behavior* (pp. 33-51). New York: Wiley.

Ehrhardt, A. A., Epstein, R., & Money, J. (1968). Fetal androgens and female gender identity in the early treated adrenogenital syndrome. *Johns Hopkins Medical Journal*, *122*, 160-167.

Ehrhardt, A. A., & Meyer-Bahlburg, H. F. (1981). Effects of prenatal sex hormones on gender-related behavior. *Science*, *211*, 1312-1318.

Elizabeth, P. H., & Green, R. (1984). Childhood sex-role behaviors: Similarities and differences in twins. *Acta Geneticae Medicae et Gemellologiae (Roma)*, *33*, 173-179.

Elkadi, S., Nicholls, M. E., & Clode, D. (1999). Handedness in opposite and same-sex dizygotic twins: Testing and testosterone hypothesis. *Neuroreport*, *10*, 333-336.

Even, M. D., Dhar, M. G., & vom Saal, F. S. (1992). Transport of steroids between fetuses via amniotic fluid in relation to the intrauterine position phenomenon in rats. *Journal of Reproduction and Fertility*, *96*, 709-716.

Fagot, B. I. (1978). The influence of sex of child on parental reactions to toddler children. *Child Development*, *49*, 459-465.

Falter, C. M., Arroyo, M., & Davis, G. J. (2006). Testosterone: Activation or organization of spatial cognition? *Biological Psychology*, *73*, 132-140.

Falter, C. M., Plaisted, K. C., & Davis, G. (2008). Visuo-spatial processing in autism-Testing the predictions of extreme male brain theory. *Journal of Autism and Developmental Disorders*, *38*, 507-515.

Fausto-Sterling, A. (1992). *Myths of gender*. New York: Basic Books.

Fein, D., Waterhouse, L., Lucci, D., Pennington, B., & Humes, M. (1985). Handedness and cognitve functions in pervasive developmental disorders. *Journal of Autism and Developmental Disorders*, *15*, 323-333.

Fels, E., & Bosch, L. R. (1971). Effect of prenatal administration of testosterone on ovarian function in rats. *American Journal of Obstetrics and gynecology*, *111*, 964-969.

Fenson, L., Dale, P. S., Reznick, J. S., Bates, E., Thal, D. J., & Pethick, S. J. (1994). Variability in early communicative development. *Monographs of the Society for Research in Child Development*, *Serial no 242, vol 59*.

Finegan, J., Bartleman, B., & Wong, P. Y. (1989). A window for the study of prenatal sex hormone influences on postnatal development. *Journal of Genetic Psychology*, *150*, 101-112.

Finkelstein, J. W., Susman, E. J., Chinchilli, V. M., Kunselman, S. J., D'Arcangelo, M. R., Schwab, J., Demers, L. M., Liben, L. S., Lookingbill, G., & Kulin, H. E. (1997). Estrogen or testosterone increases self-reported aggressive behaviors in hypogonadal adolescents. *Journal of Clinical Endocrinology and Metabolism*, *82*, 2433-2438.

Focquaert, F., Steven, M. S., Wolford, G. L., Colden, A., & Gazzaniga, M. S. (2007). Empathizing and systemizing cognitive traits in the sciences and humanities. *Personality and Individual Differences*, *43*, 619-625.

Folstein, S. E., & Rosen-Sheidley, B. (2001). Genetics of autism: Complex aetiology for a heterogeneous disorder. *Nature Reviews. Genetics*, *2*, 943-955.

Fombonne, E. (2005). The changing epidemiology of autism. *Journal of Applied Research in Intellectual Disabilities*, *18*, 281-294.

Forest, M. G., Sizonenko, P. C., Cathiard, A. M., & Bertrand, J. (1974). Hypophyso-gonadal function in humans during the first year of life: I. Evidence for testicular activity in early infancy. *The Journal of Clinical Investigation*, *53*, 819-828.

Forrest, S., Eatough, V., & Shevlin, M. (2005). Measuring adult indirect aggression: The development and psychometric assessment of the indirect aggression scales. *Aggressive Behavior*, *31*, 84-97.

Frith, U. (1991). *Autism and Asperger's Syndrome*: Cambridge University Press.

Fuchs, F., & Klopper, A. (1983). *Endocrinology of Pregnancy*. Philadelphia: Harper & Row.

Galea, L. A., & Kimura, D. (1993). Sex differences in route-learning. *Personality and Individual Differences*, *14*, 53-65.

George, F. W., & Wilson, J. D. (1992). Embryology of the Genital Tract. In P. C. Walsh, A. B. Retik & T. A. Stamey (Eds.), *Campbell's Urology 6th Ed* (pp. 1496-1508). Philadelphia: WB Saunders.

Gillberg, C. (1983). Autistic children's hand preferences: Results from an epidemiological study of infantile autism. *Psychiatry Research*, *10*, 21-30.

Gillberg, C., Nordin, V., & Ehlers, S. (1996). Early detection of autism. Diagnostic instruments for clinicians. *European Child and Adolescent Psychiatry*, *5*, 67-74.

Gitau, R., Adams, D., Fisk, N. M., & Glover, V. (2005). Fetal plasma testosterone correlates positively with cortisol. *Archives of disease in childhood. Fetal and neonatal edition*, *90*, F166-169.

Golan, O., Baron-Cohen, S., & Hill, J. (2006). The Cambridge Mindreading (CAM) Face-Voice Battery: Testing complex emotion recognition in adults with and without Asperger syndrome. *Journal of Autism and Developmental Disorders*, *36*, 169-183.

Goldenfeld, N., Baron-Cohen, S., & Wheelwright, S. (2005). Empathizing and systemizing in males, females and autism. *International Journal of Clinical Neuropsychology*, *2*, 338-345

Golombok, S., & Hines, M. (2002). Sex differences in social behavior. In P. K. Smith & C. H. Hart (Eds.), *Blackwell handbook of childhood social development* (pp. 117-136). Oxford, U.K.: Blackwell Publishers.

Golombok, S., & Rust, J. (1993a). The measurement of gender role behaviour in pre-school children: A research note. *Journal of Child Psychology and Psychiatry*, *34*, 805-811.

Golombok, S., & Rust, J. (1993b). The Pre-School Activities Inventory: A standardized assessment of gender role in children. *Psychological Assessment*, *5*, 131-136.

Goy, R. W., Bercovitch, F. B., & McBrair, M. C. (1988). Behavioral masculinization is independent of genital masculinization in prenatally androgenized female rhesus macaques. *Hormones and Behavior*, *22*, 552-571.

Goy, R. W., & McEwen, B. S. (1980). *Sexual Differentiation of the Brain*. Cambridge, MA: The MIT Press.

Granger, D. A., Shirtcliff, E. A., Booth, A., Kivlighan, K. T., & Schwartz, E. B. (2004). The "trouble" with salivary testosterone. *Psychoneuroendocrinology*, *29*, 1229-1240.

Greenberg, D. A., Hodge, S. E., Sowinski, J., & Nicoll, D. (2001). Excess of twins among affected sibling pairs with autism: Implications for the etiology of autism. *American Journal of Human Genetics*, *69*, 1062-1067.

Grimshaw, G. M., Bryden, M. P., & Finegan, J. K. (1995a). Relations between prenatal testosterone and cerebral lateralization in children. *Neuropsychology*, *9*, 68-79.

Grimshaw, G. M., Sitarenios, G., & Finegan, J. K. (1995b). Mental rotation at 7 years: Relations with prenatal testosterone levels and spatial play experiences. *Brain and Cognition*, *29*, 85-100.

Gupta, A. R., & State, M. W. (2007). Recent advances in the genetics of autism. *Biological Psychiatry*, *61*, 429-437.

Hall, J. A. Y., & Kimura, D. (1995). Sexual orientation and performance on sexually dimorphic motor tasks. *Archives of Sexual Behavior*, *24*, 395-407.

Hallmayer, J., Glasson, E. J., Bower, C., Petterson, B., Croen, L., Grether, J., & Risch, N. (2002). On the twin risk in autism. *American Journal of Human Genetics*, *71*, 941-946.

Halperin, J. M., McKay, K. E., & Newcorn, J. H. (2002). Development, reliability, and validity of the Children's Aggression Scale-Parent Version. *Journal of American Academy of Child and Adolescent Psychiatry*, *41*, 245-252.

Halpern, D. F. (1997). Sex differences in intelligence: Implications for education. *American Psychologist. Special Issue: Intelligence and Lifelong Learning*, *52*, 1091-1102.

Hamilton, A., Plunkett, K., & Shafer, G. (2000). Infant vocabulary development assessed with a British Communicative Inventory: Lower scores in the UK than the USA. *Journal of Child Language*, *27*, 689-705.

Hampson, E., Ellis, C. L., & Tenk, C. M. (2008). On the relation between 2D:4D and sex-dimorphic personality traits. *Archives of Sexual Behavior*, *37*, 133-144.

Hampson, E., Rovet, J. F., & Altmann, D. (1998). Spatial reasoning in children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Developmental Neuropsychology*, *14*, 299-320.

Harden, A. Y., Minshew, N. J., Mallikarjuhn, M., & Keshavan, M. S. (2001). Brain volume in autism. *Journal of Child Neurology*, *16*, 421-424.

Harris, J. A., Vernon, P. A., & Boomsma, D. I. (1998). The heritability of testosterone: A study of Dutch adolescent twins and their parents. *Behavior Genetics*, *28*, 165-171.

Hartin, P. J., & Barry, R. J. (1979). A comparative dermatoglyphic study of autistic, retarded, and normal children. *Journal of Autism and Developmental Disorders*, *9*, 233-246.

Henderson, B. A., & Berenbaum, S. A. (1997). Sex-typed play in opposite-sex twins. *Developmental Psychobiology*, *31*, 115-123.

Hier, D. B., & Crowley, W. F. (1982). Spatial ability in androgen-deficient men. *New England Journal of Medicine*, *306*, 1202-1205.

Hines, M. (2003). Sex steroids and human behavior: Prenatal androgen exposure and sex-typical play behavior in children. *Annals New York Academy of Sciences*, *1007*, 272-282.

Hines, M. (2004). *Brain gender*. New York, New York: Oxford University Press, Inc.

Hines, M. (2006). Prenatal testosterone and gender-related behaviour. *European Journal of Endocrinology*, *155* S115-S121.

Hines, M., Ahmed, S. F., & Hughes, I. A. (2003a). Psychological outcomes and gender-related development in complete androgen insensitivity syndrome. *Archives of Sexual Behavior*, *32*, 93-101.

Hines, M., Brook, C., & Conway, G. S. (2004). Androgen and psychosexual development: Core gender identity, sexual orientation and recalled childhood gender role behavior in women and men with congenital adrenal hyperplasia (CAH). *Journal of Sex Research*, *41*, 75-81.

Hines, M., Fane, B. A., Pasterski, V. L., Matthews, G. A., Conway, G. S., & Brook, C. (2003b). Spatial abilities following prenatal androgen abnormality: Targeting and mental rotations performance in individuals with congenital adrenal hyperplasia. *Psychoneuroendocrinology*, *28*, 1010-1026.

Hines, M., Golombok, S., Rust, J., Johnston, K. J., & Golding, J. (2002a). Testosterone during pregnancy and gender role behavior of preschool children: A longitudinal, population study. *Child Development*, *73*, 1678-1687.

Hines, M., Johnston, K. J., Golombok, S., Rust, J., Stevens, M., & Golding, J. (2002b). Prenatal stress and gender role behavior in girls and boys: A longitudinal, population study. *Hormones and Behavior*, *42*, 126-134.

Hines, M., & Shipley, C. (1984). Prenatal exposure to diethylstilbestrol (DES) and the development of sexually dimorphic cognitive abilities and cerebral lateralization. *Developmental Psychology*, *20*, 81-94.

Hoekstra, R., Bartels, M., & Boomsma, D. I. (2006). Heritability of testosterone levels in 12-year-old twins and its relation to pubertal development. *Twin Research and Human Genetics*, *9*, 558-565.

Hoekstra, R. A., Bartels, M., Cath, D. C., & Boomsma, D. I. (2008). Factor structure, reliability and criterion validity of the Autism-spectrum Quotient (AQ): A study in Dutch population and patient groups. *Journal of Autism and Developmental Disorders*.

Hoekstra, R. A., Bartels, M., Verweij, C. J., & Boomsma, D. I. (2007). Heritability of autistic traits in the general population. *Archives of Pediatric and Adolescent Medicine*, *161*, 372-377.

Holt, S. B. (1968). *The genetics of dermal ridges*. Springfield, IL: Charles C. Thomas.
Hultman, C. M., Sparen, P., & Cnattingius, S. (2002). Perinatal risk factors for infantile autism. *Epidemiology*, *13*, 417-423.
Hyde, J. S. (1984). How large are gender differences in aggression? A developmental meta-analysis. *Developmental Psychology*, *20*, 722-736.
Hyde, J. S. (2005). The gender similarities hypothesis. *American Psychologist*, *60*, 581-592.
Hyde, J. S., & Linn, M. C. (1988). Gender differences in verbal ability: A meta-analysis. *Psychological Bulletin*, *104*, 53-69.
ICD-10. (1994). *International classification of diseases* (10th ed.). Geneva, Switzerland: World Health Organisation.
Ingudomnukul, E., Baron-Cohen, S., Wheelwright, S., & Knickmeyer, R. (2007). Elevated rates of testosterone-related disorders in women with autism spectrum conditions. *Hormones and Behavior*, *51*, 597-604.
Jacklin, C. N., Maccoby, E. E., & Doering, C. H. (1983). Neonatal sex-steroid hormones and timidity in 6-18-month-old boys and girls. *Developmental Psychobiology*, *16*, 163-168.
Jacklin, C. N., Wilcox, K. T., & Maccoby, E. E. (1988). Neonatal sex-steroid hormones and cognitive abilities at six years. *Developmental Psychobiology*, *21*, 567-574.
Jarrold, C., Boucher, J., & Smith, P. (1993). Symbolic play in autism: A review. *Journal of Autism and Developmental Disorders*, *23*, 281-387.
Johnson, E. S., & Meade, A. C. (1987). Developmental patterns of spatial ability: An early sex difference. *Child Development*, *58*, 725-740.
Jolliffe, T., & Baron-Cohen, S. (1997). Are people with autism and Asperger syndrome faster than normal on the Embedded Figures Test? *Journal of Child Psychology and Psychiatry*, *38*, 527-534.
Jost, A. (1961). The role of foetal hormones in prenatal development. *Harvey Lectures*, *55*, 201-226.
Jost, A. (1970). Hormonal factors in the sex differentiation of the mammalian foetus. *Philosophical Transactions of the Royal Society of London: B Biological Sciences*, *259*, 119-130.
Jost, A. (1972). A new look at the mechanism controlling sexual differentiation in mammals. *John Hopkins Medical Journal*, *130*, 38-53.
Judd, H. L., Robinson, J. D., Young, P. E., & Jones, O. W. (1976). Amniotic fluid testosterone levels in midpregnancy. *Obstetrics and Gynecolology*, *48*, 690-692.
Kimura, D. (1999). *Sex and Cognition*. Cambridge, MA: The MIT Press.
Kimura, D., & Carson, M. W. (1995). Dermatoglyphic asymmetry: Relation to sex, handedness and cognitive pattern. *Personality and Individual Differences*, *19*, 471-478.
Kimura, D., & Clarke, P. G. (2001). Cognitive pattern and dermatoglyphic asymmetry. *Personality and Individual Differences*, *30*, 579-586.
Klin, A. (2000). Attributing social meaning to ambiguous visual stimuli in higher-functioning autism and Asperger syndrome: The Social Attribution Task. *Journal of Child Psychology and Psychiatry*, *7*, 831-846.
Knickmeyer, R., Baron-Cohen, S., Fane, B. A., Wheelwright, S., Mathews, G. A., Conway, G. S., Brook, C. G., & Hines, M. (2006a). Androgens and autistic traits: A study of individuals with congenital adrenal hyperplasia. *Hormones and Behavior*, *50*, 148-153.
Knickmeyer, R., Baron-Cohen, S., Raggatt, P., & Taylor, K. (2005a). Foetal testosterone, social relationships, and restricted interests in children. *Journal of Child Psychology and Psychiatry*, *46*, 198-210.
Knickmeyer, R., Baron-Cohen, S., Raggatt, P., Taylor, K., & Hackett, G. (2006b). Fetal testosterone and empathy. *Hormones and Behavior*, *49*, 282-292.

Knickmeyer, R., Wheelwright, S., Taylor, K., Raggatt, P., Hackett, G., & Baron-Cohen, S. (2005b). Gender-typed play and amniotic testosterone. *Developmental Psychology*, *41*, 517-528.

Knickmeyer, R. C., & Baron-Cohen, S. (2006a). Fetal testosterone and sex differences. *Early Human Development*, *82*, 755-760.

Knickmeyer, R. C., & Baron-Cohen, S. (2006b). Fetal testosterone and sex differences in typical social development and in autism. *Journal of Child Neurology*, *21*, 825-845.

Knickmeyer, R. C., Baron-Cohen, S., Auyeung, B., & Ashwin, E. (2008). How to test the extreme male brain theory of autism in terms of foetal androgens? *Journal of Autism and Developmental Disorders*, *Epub ahead of print*.

Knickmeyer, R. C., Wheelwright, S., & Baron-Cohen, S. B. (2007). Sex-typical play: Masculinization/defeminization in girls with an autism spectrum condition. *Journal of Autism and Developmental Disorders*, *Epub ahead of print*.

Knickmeyer, R. C., Wheelwright, S., Hoekstra, R., & Baron-Cohen, S. (2006c). Age of menarche in females with autism spectrum conditions. *Developmental Medicine and Child Neurology 48*, 1007-1008.

Kraemer, S. (2000). The fragile male. *British Medical Journal*, *321*, 1609-1612.

Kuepper, Y., & Hennig, J. (2007). Behavioral aggression is associated with the 2D:4D ratio in men but not women. *Journal of Individual Differences*, *28*, 64-72.

Larsen, P. R., Kronenberg, H. M., Melmed, S., & Polonsky, K. S. (Eds.). (2002). *Williams Textbook of Endocrinology* (10th ed.). Philadelphia: Saunders.

Lauritsen, M., & Ewald, H. (2001). The genetics of autism. *Acta Psychiatrica Scandinavica*, *103*, 411-427.

Lawson, J., Baron-Cohen, S., & Wheelwright, S. (2004). Empathising and systemising in adults with and without Asperger syndrome. *Journal of Autism and Developmental Disorders*, *34*, 301-310.

Lincoln, A. J., Courchesne, E., Kilman, B. A., Elmasian, R., & Allen, M. (1988). A study of intellectual abilities in high-functioning people with autism. *Journal of Autism and Developmental Disorders*, *18*, 505-524.

Linn, M. C., & Petersen, A. C. (1986). A meta-analysis of gender differences in spatial ability: Implications for mathematics and science achievement. In J. S. Hyde & M. C. Linn (Eds.), *The Psychology of Gender: Advances through Meta-Analysis*. Baltimore: Johns Hopkins University Press.

Liss, M. B. (1979). Variables influencing modeling and sex-typed play. *Psychological Reports*, *44*, 1107-1115.

Lord, C. (1995). Follow-up of two-year-olds referred for possible autism. *Journal of Child Psychology and Psychiatry*, *36*, 1365-1382.

Lord, C., Risi, S., DiLavore, P. S., Shulman, C., Thurm, A., & Pickles, A. (2006). Autism from 2 to 9 years of age. *Archives of General Psychiatry*, *63*, 694-701.

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Jr., Leventhal, B. L., DiLavore, P. C., Pickles, A., & Rutter, M. (2000). The autism diagnostic observation schedule-generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders*, *30*, 205-223.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders*, *24*, 659-685.

Lutchmaya, S., Baron-Cohen, S., & Raggatt, P. (2002a). Foetal testosterone and eye contact in 12 month old infants. *Infant Behavior and Development*, *25*, 327-335.

Lutchmaya, S., Baron-Cohen, S., & Raggatt, P. (2002b). Foetal testosterone and vocabulary size in 18- and 24-month-old infants. *Infant Behavior and Development*, *24*, 418-424.

Lutchmaya, S., Baron-Cohen, S., Raggatt, P., Knickmeyer, R., & Manning, J. T. (2004). 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Human Development*, *77*, 23-28.

Lynn, R. (1998). Sex differences in intelligence: Data from a Scottish standardisation of the WAIS-R. *Personality and Individual Differences*, *24*, 289-290.

Lynn, R., Raine, A., Venables, P. H., Mednick, S. A., & Irwing, P. (2005). Sex differences on the WISC-R in Mauritius. *Intelligence*, *33*, 527–533.

Maccoby, E. E. (1998). The two sexes: Growing up apart, coming together. *Family and public policy. Cambridge, MA, US: Belknap Press/Harvard University Press*, *376*.

Maccoby, E. E., Doering, C. H., Jacklin, C. N., & Kraemer, H. (1979). Concentrations of sex hormones in umbilical cord blood: Their relation to sex and birth order of infants. *Child Development*, *50*, 632-642.

Maccoby, E. E., & Jacklin, C. N. (1974). *The psychology of sex differences*. Stanford, CA: Stanford University Press.

Malouf, M. A., Migeon, C. J., Carson, K. A., Petrucci, L., & Wisniewski, A. B. (2006). Cognitive outcome in adult women affected by congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Hormone Research*, *65*, 142-150.

Manning, J. T. (2002). *Digit ratio: A pointer to fertility, behavior and health*. New Brunswick, NJ: Rutgers University Press.

Manning, J. T., Baron-Cohen, S., Wheelwright, S., & Sanders, G. (2001). The 2nd to 4th digit ratio and autism. *Developmental Medicine & Child Neurology*, *43*, 160-164.

Manning, J. T., & Taylor, R. P. (2001). Second to fourth digit ratio and male ability in sport: Implications for sexual selection in humans. *Evolution and Human Behavior*, *22*, 61-69.

Marcus, J., Maccoby, E. E., Jacklin, C. N., & Doering, C. H. (1985). Individual differences in mood in early childhood: their relation to gender and neonatal sex steroids. *Developmental Psychobiology*, *18*, 327-340.

Masters, M. S., & Sanders, B. (1993). Is the gender difference in mental rotation disappearing? *Behavior Genetics*, *23*, 337-341.

Matson, J. L., Dixon, D. R., & Matson, M. L. (2005). Assessment and treatment of aggressive behavior in children and adolescents with developmental disabilities. *Journal of Educational Psychology*, *25*, 151–181.

Matson, J. L., & Nebel-Schwalm, M. (2007). Assessing challenging behaviors in children with autism spectrum disorders: A review. *Research in Developmental Disabilities*, *28*, 567-579.

McClintock, K., Hall, S., & Oliver, C. (2003). Risk markers associated with challenging behaviours in people with intellectual disabilities: A meta-analytic study. *Journal of Intellectual Disability Research*, *47*, 405-416.

McManus, I. C., Murray, B., Doyle, K., & Baron-Cohen, S. (1992). Handedness in childhood autism shows a dissociation of skill and preference. *Cortex*, *28*, 373-381.

Mehrabian, A., & Epstein, N. (1972). A measure of emotional empathy. *Journal of Personality*, *40*, 525-543.

Meisel, R. L., & Ward, I. L. (1981). Fetal female rats are masculinized by male littermates located caudally in the uterus. *Science*, *213*, 239-242.

Meyer-Bahlburg, H. F. L., Erhardt, A. A., & Feldman, J. F. (1985). Questionnaires for the assessment of atypical gender role behavior: A methodological study. *Journal of the American Academy of Child Psychiatry*, *24*.

Miller, E. M., & Martin, N. (1995). Analysis of the effect of hormones on opposite-sex twin attitudes. *Acta Geneticae Medicae et Gemellologiae (Roma)*, *44*, 41-52.

Milne, E., White, S., Campbell, R., Swettenham, J., Hansen, P., & Ramus, F. (2006). Motion and form coherence detection in autistic spectrum disorder: Relationship to motor control and 2:4 digit ratio. *Journal of Autism and Developmental Disorders*, *36*, 225-237.

Money, J., Schwartz, M., & Lewis, V. (1984). Adult erotosexual status and fetal hormonal masculinization and demasculinization: 46 XX congenital adrenal hyperplasia and 46 XY androgen insensitivity syndrome compared. *Psychoneuroendocrinology*, *9*, 405-414.

Money, J., & Scwartz, M. (1976). Fetal androgens in the early treated adrenogenital syndrome of 46XX hermaphroditism: Influence on assertive and aggressive types of behavior. *Aggressive Behavior*, *2*, 19-30.

Nagamani, M., McDonough, P. G., Ellegood, J. O., & Mahesh, V. B. (1979). Maternal and amniotic fluid steroids throughout human pregnancy. *American Journal of Obstetrics and Gynecology*, *134*, 674-680.

Nass, R., Baker, S., Speiser, P., Virdis, R., Balsamo, A., Cacciari, E., Loche, A., Dumic, M., & New, M. (1987). Hormones and handedness: Left-hand bias in female adrenal hyperplasia patients. *Neurology*, *37*, 711-715.

Nebot, T. K. (1988). Sex differences among children on embedded tasks. *Perceptual and Motor Skills*, *67*, 972-974.

New, M. I. (1998). Diagnosis and management of congenital adrenal hyperplasia. *Annual Review of Medicine*, *49*, 311-328.

Nordenstrom, A., Servin, A., Bohlin, G., Larsson, A., & Wedell, A. (2002). Sex-typed toy play behavior correlates with the degree of prenatal androgen exposure assessed by the *CYP21* genotype in girls with congenital adrenal hyperplasia. *The Journal of Clinical Endocrinology and Metabolism*, *87*, 5119-5124.

Novy, M., & Resko, J. (Eds.). (1981). *Fetal Endocrinology*. New York, New York: Academic Press.

Nystul, M. S. (1981). Effects of siblings' sex composition on self-concept. *Journal of Psychology: Interdisciplinary and Applied*, *108*, 133-136.

Olweus, D., Mattsson, A., Schalling, D., & Low, H. (1980). Testosterone, aggression, physical, and personality dimensions in normal adolescent males. *Psychosomatic Medicine*, *42*, 253-269.

Pang, S., Levine, L. S., Chow, D. M., Faiman, C., & New, M. I. (1979). Serum androgen concentrations in neonates and young infants with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Clinical Endocrinology*, *11*, 575-584.

Pasterski, V., Hindmarsh, P., Geffner, M., Brook, C., Brain, C., & Hines, M. (2007). Increased aggression and activity level in 3- to 11-year-old girls with congenital adrenal hyperplasia (CAH). *Hormones and Behavior*, *52*, 368-374.

Pasterski, V. L., Geffner, M. E., Brain, C., Hindmarsh, P., Brook, C., & Hines, M. (2005). Prenatal hormones and postnatal socialization by parents as determinants of male-typical toy play in girls with congenital adrenal hyperplasia. *Child Development*, *76*, 264-278.

Paul, S. N., Kato, B. S., Cherkas, L. F., Andrew, T., & Spector, T. D. (2006). Heritability of the second to fourth digit ratio (2d:4d): A twin study. *Twin Research and Human Genetics*, *9*, 215-219.

Peters, M. (1991). Sex differences in human brain size and the general meaning of differences in brain size. *Canadian Journal of Psychology*, *45*, 507-522.

Phoenix, C. H., Goy, R. W., Gerall, A. A., & Young, W. C. (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*, *65*, 369-382.

Piven, J., Palmer, P., Jacobi, D., Childress, D., & Arndt, S. (1997). Broader autism phenotype: Evidence from a family history study of multiple-incidence autism familiies. *American Journal of Psychiatry*, *154*, 185-190.

Quadagno, D. M., Briscoe, R., & Quadagno, J. S. (1977). Effects of perinatal gonadal hormones on selected nonsexual behavior patterns: A critical assessment of the nonhuman and human literature. *Psychological Bulletin*, *84*, 62-80.

Reinisch, J. M. (1981). Prenatal exposure to synthetic progestins increases potential for agression in humans. *Science*, *211*, 1171-1173.

Reinisch, J. M., & Sanders, S. A. (1986). A test of sex differences in aggressive response to hypothetical conflict situations. *Journal of Personality and Social Psychology*, *50*, 1045-1049.

Resnick, S. M., Berenbaum, S. A., Gottesman, I. I., & Bouchard, T. J. (1986). Early hormonal influences on cognitive functioning in congenital adrenal hyperplasia. *Developmental Psychology*, *22*, 191-198.

Resnick, S. M., Gottesman, I. I., & McGue, M. (1993). Sensation seeking in opposite-sex twins: An effect of prenatal hormones? *Behavior Genetics*, *23*, 323-329.

Richman, W. L., Kiesler, S., Weisband, S., & Drasgow, F. (1999). A meta-analytic study of social desirability distortion in computer-administered questionnaires, traditional questionnaires, and interviews. *Journal of Applied Psychology*, *84*, 754-775.

Ritvo, E. R., Freeman, B. J., Mason-Brothers, A., Mo, A., & Ritvo, A. M. (1985). Concordance for the syndrome of autism in 40 pairs of afflicted twins. *American Journal of Psychiatry*, *142*, 74-77.

Robinson, J., Judd, H., Young, P., Jones, D., & Yen, S. (1977). Amniotic fluid androgens and estrogens in midgestation. *Journal of Clinical Endocrinology*, *45*, 755-761.

Rodeck, C. H., Gill, D., Rosenberg, D. A., & Collins, W. P. (1985). Testosterone levels in midtrimester maternal and fetal plasma and amniotic fluid. *Prenatal Diagnosis*, *5*, 175-181.

Rodgers, C. S., Fagot, B. I., & Winebarger, A. (1998a). Gender-typed toy play in dizygotic twin pairs: A test of hormone transfer theory. *Sex Roles*, *39*, 173-184.

Rodgers, C. S., Fagot, B. I., & Winebarger, A. (1998b). Gender-typed toy play in dizygotic twin pairs: A test of hormone transfer theory. *Sex Roles*, *39*, 173-184.

Ronald, A., Happe, F., Bolton, P., Butcher, L. M., Price, T., Wheelwright, S., Baron-Cohen, S., & Plomin, R. (2006). Genetic heterogeneity between the three components of the Autism Spectrum: A twin study. *Journal of the American Academy of Child and Adolescent Psychiatry*, *45*, 691-699.

Rönnlund, M., & Nilsson, L. (2006). Adult life-span patterns in WAIS-R Block Design performance: Cross-sectional versus longitudinal age gradients and relations to demographic factors. *Intelligence*, *34*, 63-78.

Rutter, M. (1978). Diagnosis and definition. In I. M. R. E. Schopler (Ed.), *Autism: A reappraisal of concepts and treatment* (pp. 1-26). New York: Plenum Press.

Rutter, M., Caspi, A., & Moffitt, T. E. (2003). Using sex differences in psychopathology to study causal mechanisms: Unifying issues and research strategies. *Journal of Child Psychology and Psychiatry*, *44*, 1092-1115.

Sanders, G., & Waters, F. (2001). Fingerprint asymmetry predicts within sex differences in the performance of sexually dimorphic tasks. *Personality and Individual Differences*, *31*, 1181-1191.

Sangalli, M., Langdana, F., & Thurlow, C. (2004). Pregnancy loss rate following routine genetic amniocentesis at Wellington Hospital. *New Zealand Medical Journal*, *117*, U818.

Satz, P., Soper, H., Orsini, D., Henry, R., & Zvi, J. (1985). Handedness subtypes in autism. *Psychiatric Annals*, *15*, 447-451.

Schindler, A. E. (1982). Hormones in human amniotic fluid. *Monographs on Endocrinology*, *21*, 1-158.

Scott, F., Baron-Cohen, S., Bolton, P., & Brayne, C. (2002a). Prevalence of autism spectrum conditions in children aged 5-11 years in Cambridgeshire, UK. *Autism*, *6*, 231-237.

Scott, F. J., Baron-Cohen, S., Bolton, P., & Brayne, C. (2002b). The CAST (Childhood Asperger Syndrome Test): Preliminary development of a UK screen for mainstream primary-school-age children. *Autism*, *6*, 9-13.

Servin, A., Bohlin, G., & Berlin, D. (1999). Sex differences in 1-, 3-, and 5-year-olds' toy-choice in a structured play session. *Scandinavian Journal of Psychology*, *40*, 43-48.

Servin, A., Nordenström, A., Larsson, A., & Bohlin, G. (2003). Prenatal androgens and gender-typed behavior: A study of girls with mild and severe forms of congenital adrenal hyperplasia. *Developmental Psychology*, *39*, 440-450.

Shah, A., & Frith, C. (1993). Why do autistic individuals show superior performance on the block design task? *Journal of Child Psychology and Psychiatry*, *34*, 1351-1364.

Shah, A., & Frith, U. (1983). An islet of ability in autistic children: A research note. *Journal of Child Psychology and Psychiatry*, *24*, 613-620.

Skuse, D. H. (2000). Imprinting, the X-chromosome, and the male brain: Explaining sex differences in the liability to autism. *Pediatric Research*, *47*, 9-16.

Smail, P. J., Reyes, F. I., Winter, J. S. D., & Faiman, C. (1981). The fetal hormonal environment and its effect on the morphogenesis of the genital system. In S. J. Kogan & E. S. E. Hafez (Eds.), *Pediatric Andrology* (pp. 9-19). Boston: Martinus Nijhoff.

Snow, M. E., Jacklin, C. N., & Maccoby, E. E. (1983). Sex of child differences in father-child interaction at one year of age. *Child Development*, *54*, 227-232.

Soper, H., Satz, P., Orsini, D., Henry, R., Zvi, J., & Schulman, M. (1986). Handedness patterns in autism suggest subtypes. *Journal of Autism and Developmental Disorders*, *16*, 155-167.

Spence, J. T., Helmreich, R., & J., S. (1974). The Personal Attributes Questionnaire: A measure of sex role stereotypes and masculinity-femininity. *Journal Supplement Abstract Service Catalog of Selected Documents in Psychology*, *4*, 43.

Stodgell, C. J., Ingram, J. I., & Hyman, S. L. (2001). The role of candidate genes in unraveling the genetics of autism. *International Review of Research in Mental Retardation*, *23*, 57-81.

Swettenham, J., Baron-Cohen, S., Charman, T., Cox, A., Baird, G., Drew, A., Rees, L., & Wheelwright, S. (1998). The frequency and distribution of spontaneous attention shifts between social and non-social stimuli in autistic, typically developing, and non-autistic developmentally delayed infants. *Journal of Child Psychology and Psychiatry*, *9*, 747-753.

Tanner, J. M. (1962). *Growth at adolescence: With a general consideration of the effects of hereditary and environmental factors upon growth and maturity from birth to maturity* (2nd ed.). Oxford: Blackwell.

Tidmarsh, L., & Volkmar, F. R. (2003). Diagnosis and epidemiology of autism spectrum disorders. *Canadian Journal of Psychiatry*, *48*, 517-525.

Tordjman, S., Ferrari, P., Sulmont, V., Duyme, M., & Roubertoux, P. (1997). Androgenic activity in autism. *American Journal of Psychiatry*, *154*, 1626-1627.

Tulchinsky, D., & Little, A. B. (1994). *Maternal-fetal endocrinology* (2nd ed.). Philadelphia; London: W.B. Saunders.

Udry, J. R. (2000). Biological limits of gender construction. *American Sociological Review*, *65*, 443-457.

Udry, J. R., Morris, N. M., & Kovenock, J. (1995). Androgen effects on women's gendered behaviour. *Journal of Biosocial Science*, *27*, 359-368.

van de Beek, C., Thijssen, J. H. H., Cohen-Kettenis, P. T., van Goozen, S. H., & Buitelaar, J. K. (2004). Relationships between sex hormones assessed in amniotic fluid, and maternal and umbilical cord blood: What is the best source of information to investigate the effects of fetal hormonal exposure? *Hormones and Behavior*, *46*, 663-669.

van de Beek, C., van Goozen, S. H. M., Buitelaar, J. K., & Cohen-Kettenis, P. T. (2008). Prenatal sex hormones (maternal and amniotic fluid) and gender-related play behavior in 13-month-old infants. *Archives of Sexual Behavior*, *Epub ahead of print*.

Volkmar, F. R., Stier, D. M., & Cohen, D. J. (1985). Age of recognition of pervasive developmental disorder. *American Journal of Psychiatry*, *142*, 1450-1452.

Voracek, M., & Dressler, S. G. (2006). Lack of correlation between digit ratio (2D:4D) and Baron-Cohen's "Reading the Mind in the Eyes" test, empathy, systemising, and autism-spectrum quotients in a general population sample. *Personality and Individual Differences*, *41*, 1481-1491.

Voyer, D., Voyer, S., & Bryden, M. P. (1995). Magnitude of sex differences in spatial abilities: A meta-analysis and consideration of critical variables. *Psychological Bulletin*, *117*, 250-270.

Wakabayashi, A., Baron-Cohen, S., Wheelwright, S., & Tojo, Y. (2006). The Autism-Spectrum Quotient (AQ) in Japan: A cross-cultural comparison. *Journal of Autism and Developmental Disorders*, *36*, 263-270.

Wakabayashi, A., Tojo, Y., Baron-Cohen, S., & Wheelwright, S. (2004). The Autism-Spectrum Quotient (AQ) Japanese version: Evidence from high-functioning clinical group and normal adults. *Japanese Journal of Psychology*, *75*, 78-84.

Walker, H. A. (1977). A dermatoglyphic study of autistic patients. *Journal of Autism and Childhood Schizophrenia*, *7*, 11-21.

Ward, I. L. (1977). Exogenous androgen activates female behavior in noncopulating, prenatally stressed male rats. *Journal of Comparative Physiology and Psychology*, *91*, 465-471.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence*. San Antonio, TX: The Psychological Corporation & Harcourt Brace.

Wheelwright, S., & Baron-Cohen, S. (submitted). Defining the broader, medium and narrow autism phenotype in parents of children with autism.

Wheelwright, S., Baron-Cohen, S., Goldenfeld, N., Delaney, J., Fine, D., Smith, R., Weil, L., & Wakabayashi, A. (2006). Predicting Autism Spectrum Quotient (AQ) from the Systemizing Quotient-Revised (SQ-R) and Empathy Quotient (EQ). *Brain Research*, *1079*, 47-56.

Williams, J., Scott, F., Stott, C., Allison, C., Bolton, P., Baron-Cohen, S., & Brayne, C. (2005). The CAST (Childhood Asperger Syndrome Test): Test accuracy. *Autism*, *9*, 45-68.

Williams, J., Scott, F., Stott, C., Allison, C., Bolton, P., Baron-Cohen, S., & Brayne, C. (submitted). Sex differences in social and communication skills in primary school aged children.

Williams, S. T., Conger, K. J., & Blozis, S. A. (2007). The development of interpersonal aggression during adolescence: The importance of parents, siblings, and family economics. *Child Development*, *78*, 1526-1542.

Wing, L. (1988). The Autistic Continuum. In L. Wing (Ed.), *Aspects of Autism: Biological research*. London: Gaskell/Royal College of Psychiatrists.

Witelson, S. F. (1991). Sex-differences in neuroanatomical changes with aging. *New England Journal of Medical*, *325*, 211-212.

Witkin, H. A., Dyk, R. B., Fattuson, H. F., Goodenough, D. R., & Karp, S. A. (1962). Psychological differentiation: Studies of development. *Oxford, England: Wiley*, *418*.

Witkin, H. A., Oltman, P. K., Raskin, E., & Karp, S. (1971). *A Manual for the Embedded Figures Test*. California: Consulting Psychology Press.

Woodbury-Smith, M. R., Robinson, J., Wheelwright, S., & Baron-Cohen, S. (2005). Screening adults for Asperger Syndrome using the AQ: A preliminary study of its diagnostic validity in clinical practice. *Journal of Autism and Developmental Disorders*, *35*, 331-335.

Appendix 1 – The Pre-School Activities Inventory (PSAI)

ACTIVITIES INVENTORY

This inventory is about everyday activities of children. It is in three sections: toy preferences, activities and characteristics. Each question asks how frequently the child plays with particular toys, engages in particular activities or shows particular characteristics. There are five possible answers: Never, Hardly Ever, Sometimes, Often, or Very Often. Answer each question by circling the response which best describes your child.

*This is for children 2 – 7 years of age. If your child is now **older** than 7, please think back and recall his/her behaviour at a younger age (2 – 7 years).

Please answer all of the questions. If you are unsure about which response best describes your child for any of the questions then please answer according to the response which seems most appropriate.

*Your Name: _____

*Your Child's Name: _____

Part 1: TOYS: Please answer these questions according to how often the child played with the following toys during the past month.

	Never	Hardly Ever	Some-times	Often	Very Often
1. Guns (or used objects as guns)	1	2	3	4	5
2. Jewellery	1	2	3	4	5
3. Tool Set	1	2	3	4	5
4. Dolls, doll's clothes or doll's carriage	1	2	3	4	5
5. Trains, cars or airplanes	1	2	3	4	5
6. Swords (or used objects as swords)	1	2	3	4	5
7. Tea set	1	2	3	4	5

PLEASE TURN OVER

Part 2: ACTIVITIES: Please answer these questions according to how often the child engaged in the following activities during the past month.

	Never	Hardly Ever	Some-times	Often	Very Often
1. Playing house (e.g. cleaning, cooking)	1	2	3	4	5
2. Playing with girls	1	2	3	4	5
3. Pretending to be a female character (e.g. princess)	1	2	3	4	5
4. Playing at having a male occupation (e.g. soldier)	1	2	3	4	5
5. Fighting	1	2	3	4	5
6. Pretending to be a family character (e.g. parent)	1	2	3	4	5
7. Sports and ball games	1	2	3	4	5
8. Climbing (e.g. fences, trees, gym equipment)	1	2	3	4	5
9. Playing at taking care of babies	1	2	3	4	5
10. Showing interest in real cars, trains and airplanes	1	2	3	4	5
11. Dressing up in girlish clothes	1	2	3	4	5

Part 3: CHARACTERISTICS: Please answer these questions according to how often the child showed the following characteristics during the past month.

	Never	Hardly Ever	Some-times	Often	Very Often
1. Likes to explore new surroundings	1	2	3	4	5
2. Enjoys rough-and-tumble play	1	2	3	4	5
3. Shows interest in snakes, spiders or insects	1	2	3	4	5
4. Avoids getting dirty	1	2	3	4	5
5. Likes pretty things	1	2	3	4	5
6. Avoids taking risks	1	2	3	4	5

PLEASE CHECK THAT YOU HAVE ANSWERED ALL THE QUESTIONS

THANK YOU FOR YOUR TIME AND HELP!

Appendix 2 – The Bem Sex Role Inventory (BSRI)

Children's Personality Questionnaire

Instructions: Rate your child on each item, on a scale from 1 (never or almost never true) to 7 (almost always true) by circling the appropriate number.

		never							always
1	self reliant	0	1	2	3	4	5	6	7
2	yielding	0	1	2	3	4	5	6	7
3	helpful	0	1	2	3	4	5	6	7
4	defends own beliefs	0	1	2	3	4	5	6	7
5	cheerful	0	1	2	3	4	5	6	7
6	moody	0	1	2	3	4	5	6	7
7	independent	0	1	2	3	4	5	6	7
8	shy	0	1	2	3	4	5	6	7
9	conscientious	0	1	2	3	4	5	6	7
10	athletic	0	1	2	3	4	5	6	7
11	affectionate	0	1	2	3	4	5	6	7
12	theatrical	0	1	2	3	4	5	6	7
13	assertive	0	1	2	3	4	5	6	7
14	flatterable	0	1	2	3	4	5	6	7
15	happy	0	1	2	3	4	5	6	7
16	strong personality	0	1	2	3	4	5	6	7
17	loyal	0	1	2	3	4	5	6	7
18	unpredictable	0	1	2	3	4	5	6	7
19	forceful	0	1	2	3	4	5	6	7
20	feminine	0	1	2	3	4	5	6	7
21	reliable	0	1	2	3	4	5	6	7
22	analytical	0	1	2	3	4	5	6	7
23	sympathetic	0	1	2	3	4	5	6	7
24	jealous	0	1	2	3	4	5	6	7
25	leadership ability	0	1	2	3	4	5	6	7
26	sensitive to other's needs	0	1	2	3	4	5	6	7
27	truthful	0	1	2	3	4	5	6	7
28	willing to take risks	0	1	2	3	4	5	6	7
29	understanding	0	1	2	3	4	5	6	7
30	secretive	0	1	2	3	4	5	6	7
31	makes decisions easily	0	1	2	3	4	5	6	7
32	compassionate	0	1	2	3	4	5	6	7
33	sincere	0	1	2	3	4	5	6	7
34	self-sufficient	0	1	2	3	4	5	6	7
35	eager to soothe hurt feelings	0	1	2	3	4	5	6	7

36	conceited	0	1	2	3	4	5	6	7
37	dominant	0	1	2	3	4	5	6	7
38	soft spoken	0	1	2	3	4	5	6	7
39	likable	0	1	2	3	4	5	6	7
40	masculine	0	1	2	3	4	5	6	7
41	warm	0	1	2	3	4	5	6	7
42	solemn	0	1	2	3	4	5	6	7
43	willing to take a stand	0	1	2	3	4	5	6	7
44	tender	0	1	2	3	4	5	6	7
45	friendly	0	1	2	3	4	5	6	7
46	aggressive	0	1	2	3	4	5	6	7
47	gullible	0	1	2	3	4	5	6	7
48	inefficient	0	1	2	3	4	5	6	7
49	acts as a leader	0	1	2	3	4	5	6	7
50	childlike	0	1	2	3	4	5	6	7
51	adaptable	0	1	2	3	4	5	6	7
52	individualistic	0	1	2	3	4	5	6	7
53	uses harsh language	0	1	2	3	4	5	6	7
54	unsystematic	0	1	2	3	4	5	6	7
55	competitive	0	1	2	3	4	5	6	7
56	loves children	0	1	2	3	4	5	6	7
57	tactful	0	1	2	3	4	5	6	7
58	ambitious	0	1	2	3	4	5	6	7
59	gentle	0	1	2	3	4	5	6	7
60	conventional	0	1	2	3	4	5	6	7

Thank you for your time and help!

Child's Name: _____ Child's Date of Birth: _____

Appendix 3 – The Child Behaviour Checklist-Aggression Subscale (CBC-A)

Please tick the appropriate box that best describes your child

	Not True (as far as you know)	Somewhat or Sometimes True	Very True or Often True
1. Argues a lot			
2. Cruel to animals			
3. Cruelty, bullying, or meanness to other			
4. Demands a lot of attention			
5. Destroys his/her own things			
6. Destroys things belonging to his/her family			
7. Disobedient at home			
8. Disobedient at school			
9. Gets in many fights			
10. Physically attacks people			
11. Screams a lot			
12. Stubborn, sullen, or irritable			
13. Sudden changes in mood or feelings			
14. Sulks a lot			
15. Suspicious			
16. Teases a lot			
17. Temper tantrums or hot temper			
18. Threatens people			
19. Unusually loud			
20. Please write in any problems that were not listed above:			

Appendix 4 – The Children's Aggression Scale-Parent Version (CAS-P)

Please tick the appropriate box

During the past year, how often has your child:

	Never	Once a month or less	Once a week or less	2-3 times a week	Most Days
1. snapped or yelled at children living in the home?					
2. snapped or yelled at adults living in the home?					
3. snapped or yelled at peers/friends who do not live in the home?					
4. snapped or yelled at adults who do not live in the home?					
5. cursed or sworn at children who live in the home?					
6. cursed or sworn at adults who live in the home?					
7. cursed or sworn at peers/friends who do not live in the home?					
8. cursed or sworn at adults who do not live in the home?					
9. verbally threatened to hit a child who lives in the home?					
10. verbally threatened to hit an adult who lives in the home?					
11. verbally threatened to hit peers/friends who do not live in the home?					
12. verbally threatened to hit adults who do not live in the home?					
13. slammed a door, kicked a chair, thrown or broken objects when angry?					
14. vandalized or destroyed someone else's property?					
15. taunted or teased or annoyed a pet or other animal?					

16. injured or tortured a pet or other living animal?					
17. fought with another child who lives in the home when provoked?					
18. fought with an adult who lives in the home when provoked?					
19. fought with peers/friends when provoked?					
20. fought with other adults who do not live in the home when provoked?					
21. how often did these fights result in mild physical injury (e.g. bumps and bruises)?					
22. how often did these fights result in serious physical injury (e.g. stitches, broken bones, or requiring a doctor's attention)?					
23. started a physical fight with a child who lives in the home?					
24. started a physical fight with an adult who lives in the home?					
25. started a physical fight with peers/friends who do not live in the home?					
26. started a physical fight with adults who do not live in the home?					
27. how often did these fights result in mild physical injury (e.g. bumps and bruises)?					
28. how often did these fights result in serious physical injury (e.g., stitches, broken bones, or requiring a doctor's attention)?					

Appendix 5 – The Child EQ and Child SQ (Combined)

Please complete by ticking the appropriate box for each statement

		Definitely Agree	Slightly Agree	Slightly Disagree	Definitely Disagree
1.	My child likes to look after other people.				
2.	My child often doesn't understand why some things upset other people so much.				
3.	My child doesn't mind if things in the house are not in their proper place.				
4.	My child would not cry or get upset if a character in a film died.				
5.	My child enjoys arranging things precisely (e.g. flowers, books, music collections).				
6.	My child is quick to notice when people are joking.				
7.	My child enjoys cutting up worms, or pulling the legs off insects.				
8.	My child is interested in the different members of a specific animal category (e.g. dinosaurs, insects, etc).				
9.	My child has stolen something they wanted from their sibling or friend.				
10.	My child is interested in different types of vehicles (e.g. types of trains, cars, planes etc).				
11.	My child does not spend large amounts of time lining things up in a particular order (e.g. toy soldiers, animals, cars).				
12.	If they had to build a Lego or Meccano model, my child would follow an instruction sheet rather than "ploughing straight in".				
13.	My child has trouble forming friendships.				
14.	When playing with other children, my child spontaneously takes turns and shares toys.				
15.	My child prefers to read or listen to fiction rather than non-fiction.				
16.	My child's bedroom is usually messy rather than organised.				
17.	My child can be blunt giving their opinions, even when these may upset someone.				
18.	My child would enjoy looking after a pet.				
19.	My child likes to collect things (e.g. stickers, trading cards, etc).				

20.	My child is often rude or impolite without realizing it.				
21.	My child knows how to mix paints to produce different colours.				
22.	My child would not notice if something in the house had been moved or changed.				
23.	My child has been in trouble for physical bullying.				
24.	My child enjoys physical activities with set rules (e.g. martial arts, gymnastics, ballet, etc).				
25.	My child can easily figure out the controls of the video or DVD player.				
26.	At school, when my child understands something they can easily explain it clearly to others.				
27.	My child would find it difficult to list their top 5 songs or films in order.				
28.	My child has one or two close friends, as well as several other friends.				
29.	My child quickly grasps patterns in numbers in math.				
30.	My child listens to others' opinions, even when different from their own.				
31.	My child shows concern when others are upset.				
32.	My child is not interested in understanding the workings of machines (e.g. cameras, traffic lights, the TV, etc).				
33.	My child can seem so preoccupied with their own thoughts that they don't notice others getting bored.				
34.	My child enjoys games that have strict rules (e.g. chess, dominos, etc).				
35.	My child gets annoyed when things aren't done on time.				
36.	My child blames other children for things that they themselves have done.				
37.	My child gets very upset if they see an animal in pain.				
38.	My child knows the differences between the latest models of games-consoles (e.g. X-box, Playstation, Playstation 2, etc.,) or other gadgets.				
39.	My child remembers large amounts of information about a topic that interests them (e.g. flags of the world, football teams, pop groups, etc).				
40.	My child sometimes pushes or pinches someone if they are annoying them.				
41.	My child is interested in following the route on a map on a journey.				
42.	My child can easily tell when another				

	person wants to enter into conversation with them.				
43.	My child is good at negotiating for what they want.				
44.	My child likes to create lists of things (e.g. favorite toys, TV programs, etc).				
45.	My child would worry about how another child would feel if they weren't invited to a party.				
46.	My child likes to spend time mastering particular aspects of their favorite activities (e.g. skate-board or yo-yo tricks, football or ballet moves).				
47.	My child finds using computers difficult.				
48.	My child gets upset at seeing others crying or in pain.				
49.	If they had a sticker album, my child would not be satisfied until it was completed.				
50.	My child enjoys events with organized routines (e.g. brownies, cubs, beavers, etc).				
51.	My child is not bothered about knowing the exact timings of the day's plans.				
52.	My child likes to help new children integrate in class.				
53.	My child has been in trouble for name-calling or teasing.				
54.	My child would not enjoy working to complete a puzzle (e.g. crossword, jigsaw, word-search).				
55.	My child tends to resort to physical aggression to get what they want.				

Appendix 6 – The Autism Spectrum Quotient- Children's Version (AQ-Child)

Please answer each of the following questions about your child or the person who is under your care by ticking a box that reflects your answer to the question most appropriately. If there is any question that you feel not able to comment, please ask your son, daughter, partner or the person to answer.

	Definitely Agree	Slightly Agree	Slightly Disagree	Definitely Disagree
1. S/he prefers to do things with others rather than on her/his own.				
2. S/he prefers to do things the same way over and over again.				
3. If s/he tries to imagine something, s/he finds it very easy to create a picture in her/his mind.				
4. S/he frequently gets so strongly absorbed in one thing that s/he loses sight of other things.				
5. S/he often notices small sounds when others do not.				
6. S/he usually notices house numbers or similar strings of information.*				
7. S/he has difficulty understanding rules for polite behaviour. *				
8. When s/he is read a story, s/he can easily imagine what the characters might look like. *				
9. S/he is fascinated by dates.				
10. In a social group, s/he can easily keep track of several different people's conversations.				
11. S/he finds social situations easy.				
12. S/he tends to notice details that others do not.				
13. S/he would rather go to a library than a birthday party. *				
14. S/he finds making up stories easy.				
15. S/he is drawn more strongly to people than to things. *				
16. S/he tends to have very strong interests, which s/he gets upset about if s/he can't pursue.				
17. S/he enjoys social chit-chat.				

18. When s/he talks, it isn't always easy for others to get a word in edgeways.				
19. S/he is fascinated by numbers.				
20. When s/he is read a story, s/he finds it difficult to work out the characters' intentions or feelings. *				
21. S/he doesn't particularly enjoy fictional stories. *				
22. S/he finds it hard to make new friends.				
23. S/he notices patterns in things all the time.				
24. S/he would rather go to the cinema than a museum. *				
25.It does not upset him/her if his/her daily routine is disturbed.				
26. S/he doesn't know how to keep a conversation going with her/his peers. *				
27. S/he finds it easy to "read between the lines" when someone is talking to her/him.				
28. S/he usually concentrates more on the whole picture, rather than the small details.				
29. S/he is not very good at remembering phone numbers.				
30. S/he doesn't usually notice small changes in a situation, or a person's appearance.				
31. S/he knows how to tell if someone listening to him/her is getting bored.				
32. S/he finds it easy to go back and forth between different activities. *				
33. When s/he talk on the phone, s/he is not sure when it's her/his turn to speak.				
34. S/he enjoys doing things spontaneously.				
35. S/he is often the last to understand the point of a joke.				
36. S/he finds it easy to work out what someone is thinking or feeling just by looking at their face.				
37. If there is an interruption, s/he can switch back to what s/he was doing very quickly.				
38. S/he is good at social chit-chat.				
39. People often tell her/him that s/he keeps going on and on about the same				

40. When s/he was in preschool, s/he used to enjoy playing games involving pretending with other children. *				
41. S/he likes to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).				
42. S/he finds it difficult to imagine what it would be like to be someone else.				
43. S/he likes to plan any activities s/he participates in carefully.				
44. S/he enjoys social occasions.				
45. S/he finds it difficult to work out people's intentions.				
46. New situations make him/her anxious.				
47. S/he enjoys meeting new people.				
48. S/he is good at taking care not to hurt other people's feelings. *				
49. S/he is not very good at remembering people's date of birth.				
50. S/he finds it very to easy to play games with children that involve pretending.				

Note: Aside from the self-report to parent-report format change, items were changed as little as possible. *Denotes items that were changed substantially.

Appendix 7 – The Childhood Autism Spectrum Test (CAST)

Please read the following questions carefully, and circle the appropriate answer. All responses are confidential.

1.	Does s/he join in playing games with other children easily?	Yes	No
2.	Does s/he come up to you spontaneously for a chat?	Yes	No
3.	Was s/he speaking by 2 years old?	Yes	No
4.	Does s/he enjoy sports?	Yes	No
5.	Is it important to him/her to fit in with the peer group?	Yes	No
6.	Does s/he appear to notice unusual details that others miss?	Yes	No
7.	Does s/he tend to take things literally?	Yes	No
8.	When s/he was 3 years old, did s/he spend a lot of time pretending (e.g., play-acting being a superhero, or holding teddy's tea parties)?	Yes	No
9.	Does s/he like to do things over and over again, in the same way all the time?	Yes	No
10	Does s/he find it easy to interact with other children?	Yes	No
11	Can s/he keep a two-way conversation going?	Yes	No
12	Can s/he read appropriately for his/her age?	Yes	No
13	Does s/he mostly have the same interests as his/her peers?	Yes	No
14	Does s/he have an interest which takes up so much time that s/he does little else?	Yes	No
15	Does s/he have friends, rather than just acquaintances?	Yes	No
16	Does s/he often bring you things s/he is interested in to show you?	Yes	No
17	Does s/he enjoy joking around?	Yes	No
18	Does s/he have difficulty understanding the rules for polite behaviour?	Yes	No
19	Does s/he appear to have an unusual memory for details?	Yes	No

20	Is his/her voice unusual (e.g., overly adult, flat, or very monotonous)?	Yes	No
21	Are people important to him/her?	Yes	No
22	Can s/he dress him/herself?	Yes	No
23	Is s/he good at turn-taking in conversation?	Yes	No
24	Does s/he play imaginatively with other children, and engage in role-play?	Yes	No
25	Does s/he often do or say things that are tactless or socially inappropriate?	Yes	No
26	Can s/he count to 50 without leaving out any numbers?	Yes	No
27	Does s/he make normal eye-contact?	Yes	No
28	Does s/he have any unusual and repetitive movements?	Yes	No
29	Is his/her social behaviour very one-sided and always on his/her own terms?	Yes	No
30	Does s/he sometimes say "you" or "s/he" when s/he means "I"?	Yes	No
31	Does s/he prefer imaginative activities such as play-acting or story-telling, rather than numbers or lists of facts?	Yes	No
32	Does s/he sometimes lose the listener because of not explaining what s/he is talking about?	Yes	No
33	Can s/he ride a bicycle (even if with stabilisers)?	Yes	No
34	Does s/he try to impose routines on him/herself, or on others, in such a way that it causes problems?	Yes	No
35	Does s/he care how s/he is perceived by the rest of the group?	Yes	No
36	Does s/he often turn conversations to his/her favourite subject rather than following what the other person wants to talk about?	Yes	No
37	Does s/he have odd or unusual phrases?	Yes	No

OTHER MEDICAL CONDITIONS/SPECIAL NEEDS

Please complete as appropriate

38 Have teachers/health visitors ever expressed any concerns about his/her development? Yes No

If Yes, please specify

39 Has s/he ever been diagnosed with any of the following?

a) Language delay Yes No

b) Hyperactivity/Attention Deficit Disorder (ADHD) Yes No

c) Dyspraxia Yes No

d) Hearing or visual difficulties Yes No

e) Autism Spectrum Condition, including Asperger Syndrome Yes No

f) A physical disability Yes No

g) A medical condition that affects development (eg Down's syndrome, chromosomal abnormality etc) Yes No

h) Other (please specify) Yes No

40 Has your child ever had febrile convulsions or febrile seizures? Yes No

41 Has your child ever had other types of seizures, fits, faints or turns? Yes No

42 Has your child been diagnosed as having epilepsy? Yes No

42A If YES, did your child have a seizure during the last two years? Yes No

42B If YES, does your child currently receive anticonvulsant drugs? Yes No

Please list the names of any anticonvulsant drugs your child is currently taking

Appendix 8 – The Quantitative Checklist for Autism in Toddlers

Section 1. Please answer the following questions about your child. Try to answer every question if you can.

1. Does you child look at you when you call his/her name?
- □ always
- □ usually
- □ sometimes
- □ rarely
- □ never

2. How easy is it for you to get eye contact with your child?
- □ very easy
- □ quite easy
- □ quite difficult
- □ very difficult
- □ impossible

3. When your child is playing alone, does s/he line objects up?
- □ always
- □ usually
- □ sometimes
- □ rarely
- □ never

4. Can other people easily understand your child's speech?
- □ always
- □ usually
- □ sometimes
- □ rarely
- □ never
- □ my child does not speak

5. Does your child point to indicate that s/he wants something (eg a toy that is out of reach)
- □ many times a day
- □ a few times a day
- □ a few times a week
- □ less than once a week
- □ never

6. Does you child point to share interest with you (eg pointing at an interesting sight)?
- □ many times a day
- □ a few times a day
- □ a few times a week
- □ less than once a week
- □ never

7. How long can your child's interest be maintained by a spinning object (eg washing machine, electric fan, toy car wheels)?

- ☐ several hours
- ☐ half an hour
- ☐ ten minutes
- ☐ a couple of minutes
- ☐ less than a minute

8. How many words can your child say?

- ☐ none – s/he has not started speaking yet
- ☐ less than 10 words
- ☐ 10 – 50 words
- ☐ 51 – 100 words
- ☐ over 100 words

9. Does your child pretend (eg care for dolls, talk on a toy phone)?

- ☐ many times a day
- ☐ a few times a day
- ☐ a few times a week
- ☐ less than once a week
- ☐ never

10. Does your child follow where you're looking?

- ☐ many times a day
- ☐ a few times a day
- ☐ a few times a week
- ☐ less than once a week
- ☐ never

11. How often does your child sniff or lick unusual objects?

- ☐ many times a day
- ☐ a few times a day
- ☐ a few times a week
- ☐ less than once a week
- ☐ never

12. Does your child place your hand on an object when s/he wants you to use it (eg on a door handle when s/he wants you to open the door, on a toy when she wants you to activate it)?

- ☐ many times a day
- ☐ a few times a day
- ☐ a few times a week
- ☐ less than once a week
- ☐ never

13. Does your child walk on tiptoe?
- □ always
- □ usually
- □ sometimes
- □ rarely
- □ never

14. How easy is it for your child to adapt when his/her routine changes or when things are out of their usual place?
- □ very easy
- □ quite easy
- □ quite difficult
- □ very difficult
- □ impossible

15. If you or someone else in the family is visibly upset, does your child show signs of wanting to comfort them? (eg stroking their hair, hugging them)?
- □ always
- □ usually
- □ sometimes
- □ rarely
- □ never

16. Does your child do the same thing over and over again (eg running the tap, turning the light switch on and off, opening and closing doors)?
- □ many times a day
- □ a few times a day
- □ a few times a week
- □ less than once a week
- □ never

17. Would you describe your child's first words as:
- □ very typical
- □ quite typical
- □ slightly unusual
- □ very unusual
- □ my child doesn't speak

18. Does your child echo things s/he hears (eg things that you say, lines from songs or movies, sounds)?
- □ many times a day
- □ a few times a day
- □ a few times a week
- □ less than once a week
- □ never

19. Does your child use simple gestures (eg wave goodbye)?
- ☐ many times a day
- ☐ a few times a day
- ☐ a few times a week
- ☐ less than once a week
- ☐ never

20. Does your child make unusual finger movements near his/her eyes?
- ☐ many times a day
- ☐ a few times a day
- ☐ a few times a week
- ☐ less than once a week
- ☐ never

21. Does your child spontaneously look at your face to check your reaction when faced with something unfamiliar?
- ☐ always
- ☐ usually
- ☐ sometimes
- ☐ rarely
- ☐ never

22. How long can your child's interest be maintained by just one or two objects?
- ☐ most of the day
- ☐ several hours
- ☐ half an hour
- ☐ ten minutes
- ☐ a couple of minutes

23. Does your child twiddle objects repetitively (eg pieces of string)?
- ☐ many times a day
- ☐ a few times a day
- ☐ a few times a week
- ☐ less than once a week
- ☐ never

24. Does your child seem oversensitive to noise?
- ☐ always
- ☐ usually
- ☐ sometimes
- ☐ rarely
- ☐ never

25. Does your child stare at nothing with no apparent purpose?
- ☐ many times a day
- ☐ a few times a day
- ☐ a few times a week
- ☐ less than once a week
- ☐ never

26a. Has you or any other person ever expressed any concerns about your child's development?
Yes No

26b. If Yes, please specify

26c. If yes, who was the person who first raised concerns about your child?

- ☐ parent
- ☐ other family member/friend
- ☐ health visitor
- ☐ GP
- ☐ other health professional

26d. If yes, how old was your child when these concerns were FIRST raised?

Months old