How is Sex Related to Autism?

Meng-Chuan Lai

Girton College

University of Cambridge

This dissertation is submitted for the degree of

Doctor of Philosophy

August 2011

Preface

The works in this dissertation were carried out at the Autism Research Centre, Department of Psychiatry, University of Cambridge between October 2008 and August 2011, and was supported by funding from the Ministry of Education, Taiwan. Professor Simon Baron-Cohen acted as my primary supervisor and Professor John Suckling acted as co-supervisor. The dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration, except that the recruitment and testing of the male participants were carried out between July 2007 and November 2008 by the Medical Research Council Autism Imaging Multicentre Study (MRC AIMS) Consortium project. This dissertation is less than 60,000 words.

Chapter 2 and parts of Chapter 1 are published in:

Meng-Chuan Lai, Michael V. Lombardo, Greg Pasco, Amber N. V. Ruigrok, Sally J. Wheelwright, Susan A. Sadek, Bhismadev Chakrabarti, MRC AIMS Consortium and Simon Baron-Cohen. (2011). A behavioral comparison of male and female adults with high functioning autism spectrum conditions. *PLoS ONE* 6(6):e20835.

Chapter 6 Study 1 is published in:

Meng-Chuan Lai, Michael V. Lombardo, Bhismadev Chakrabarti, Susan A. Sadek, Greg Pasco, Sally J. Wheelwright, Edward T. Bullmore, Simon Baron-Cohen, MRC AIMS Consortium and John Suckling. (2010). A shift to randomness of brain oscillations in people with autism. *Biological Psychiatry* 68(12):1092-1099.

Chapter 3 is currently in preparation for publication as:

Meng-Chuan Lai, Michael V. Lombardo, Amber N. V. Ruigrok, Bhismadev Chakrabarti, Sally J. Wheelwright, MRC AIMS Consortium and Simon Baron-Cohen. (in preparation). Sex-general and sex-specific cognition in adults with high-functioning autism spectrum conditions.

Chapters 4 and 5 are currently in preparation for publication as:

Meng-Chuan Lai, Michael V. Lombardo, Amber N. V. Ruigrok, Bhismadev Chakrabarti, John Suckling, Edward T. Bullmore, MRC AIMS Consortium and Simon Baron-Cohen. (in preparation). Do women with autism have an 'extreme male brain'?

Chapter 6 Study 2 is in preparation for publication as:

Meng-Chuan Lai, Michael V. Lombardo, Bhismadev Chakrabarti, Amber N. V. Ruigrok, Edward T. Bullmore, Simon Baron-Cohen, MRC AIMS Consortium and John Suckling. (in preparation). Differential complexity of resting brain oscillations between sexes and in autism spectrum conditions.

Acknowledgements

I am extremely grateful for my supervisors who contributed so much to my maturation as a scientist. Professor Simon Baron-Cohen, he himself being the main reason why I chose to pursue my PhD at Cambridge, has always been extraordinarily supportive, generous, trustful and patient, meanwhile enlightening and inspirational in nurturing my academic development. His wisdom, vision and elegant viewpoints in psychology, autism research and cognitive neuroscience have always been the most helpful guidance for me as a freshman to the research field. With Simon's tremendous supports I find the doctoral study very rewarding and enjoyable. Simon will always be my role model – the best mentor one can ever imagine. Professor John Suckling guided me to study neuroimaging and complex science from scratch, and helped me get over my (irrational) worries in learning in this completely new field at the beginning of my study. John is always constructively critical and at the same time extremely supportive and helpful. Not a single meeting with him do I not feel enlightened and empowered. I always come out of his office full of joy and courage, 'charged' by his creativity, optimism and sharp viewpoints. John teaches me how to do science wholeheartedly but constantly keep a clear 'metacognition'. I believe I will benefit from this for life.

I would also like to thank my advisors who have provided immense supports and guidance. I cannot be luckier to work with Michael Lombardo so closely throughout the whole PhD. Mike is always promptly responsive, extremely helpful, inspirational and supportive. He taught me everything an imager should know from scratch, guided me through the most basic (but boring for an experienced person!) bits of

programming, and reviewed and commented on my very first immature drafts of each single manuscript I wrote. The most important thing I learned from him, probably owing to his 'American spirit', is not hesitate to try and learn new things and face novel challenges. I used to think too much but do too little – Mike showed me how to achieve a balance in between. Without Mike I would not have the opportunity to learn the interesting themes in social neuroscience, and the various imaging methodologies that I have applied. I wholeheartedly thank him for all his supports. I am also very fortunate in having Bhismadev Chakrabarti enlightening me for nearly every aspect in autism research, from genetics to behaviour. Bhisma is a renaissance man. I benefit so much from his invariably insightful comments based on his deep and broad, focused but integrative knowledge across genetics, biology, statistics, imaging, affective and social neuroscience. Bhisma set the standard for me of what a 'Doctor of Philosophy' should be like. Lastly, I am so honoured to have Professor Ed Bullmore advising me in learning complex science and graph theoretical network analysis. Ed has the amazingly clear mind and sharp eyes to see both the nuance within a study and how it is significant to the whole field. His advices and critiques always bring me up to a higher level to examine the works, which I think is the most precious experience one can ever have on working with such a great figure like him. I am also very grateful for my examiners Professor Digby Tantam, Drs. Graham Murray and Howard Ring for their insightful critiques and suggestions to my research and this dissertation.

My major project for the dissertation, the Female Autism Imaging Multicentre Study (Female AIMS), is not attainable without the great helps from co-researchers. I am most grateful to Amber Ruigrok for her extraordinary contributions in realising all projects, all the way from preparation, recruitment, testing to data organisation. It is a

great pleasure to work together with Amber. I would also like to express my gratitude to Renate van de Ven, Elisa Scheller and Mohammad Lone for their significant contributions in helping out the relevant projects. Most importantly, I sincerely appreciate the enthusiastic and generous volunteering and offering to help of all our participants. It is their contributions that push me (and the field) forward.

My sincere gratitude also goes to several senior colleagues at the Autism Research Centre (ARC) who have helped me through a variety of challenges during the past years. Bonnie Auyeung's always warm and genuine concerns are great supports to me in the long journey pursuing my degree. I especially thank her for the prompt helps in commenting on some of my chapters. Sally Wheelwright's sharp discussions and critical eyes to science are still influential to me up to now even though she has left the lab. I miss her bright and straightforward characteristics very much. Greg Pasco's generous and warm supports to the research project cannot be overemphasised. I especially miss his fantastic British humour! Carrie Allison's careful looking after the ethical aspects of the project and the great helps in data cleaning and organisation are pivotal to the smooth completion of the projects. Rosa Hoekstra is my best consultant in statistics and has provided many wonderful suggestions. Liliana Ruta's passion in science and rich clinical experiences have substantially inspired me, and yes, we will certainly gather together sometime in the 'academic house' at Taormina! I would also like to thank Teresa Tavassoli for her constant 'unconditional positive regards' and supports to all aspects of my research.

I am so grateful for all lab mates in the lovely ARC, who nurtured my academic perspectives in the broad field of autism research. Thank you all Mike Spencer, Gina Gómez, Jill Sullivan, Jon Breidbord, Alexia Rattazzi, Kimberly Armstrong, Lindsay

Chura, Caroline Robertson, Ed Sucksmith, Susan Sadek, Rosie Holt, Sophia Sun, Chris and Emma Ashwin, Ofer Golan and Matthew Belmonte. Particular thank goes to Fiona Scott for training me on administering the ADI-R and ADOS and sharing her rich clinical experiences and perspectives. The administrative colleagues also have provided immense supports and assistances, both administratively and personally. I would like to express my sincere gratitude to all of you – Gaenor Moore, Paula Niami, Rachel Jackson, Jenny Hannah and Chloe Gayer-Anderson.

The colleagues at the Brain Mapping Unit are my strong backup whenever I am in trouble with trivial but crucial issues in neuroimaging – thank you Cinly Ooi, Anna Barnes and Julian Henty. I particularly want to thank a group of colleagues who have led me into the excitingly interesting field of complex network science and have guided me all the way from the very basics: Dani Bassett, Alex Fornito, David Meunier, Andrew Zalesky, Petra Vertes and Aaron Alexander-Bloch.

My PhD is established on the achievements of the (male) MRC AIMS project. I am very grateful for the MRC, the consortium and all the researchers from London, Oxford and Cambridge sites that provide me with the resources and the invaluable data to build on and to establish my own projects. Particular appreciation goes to Christine Ecker and Professor Declan Murphy for their generous supports. I would also like to thank Michael Craig, Flavio Dell' Acqua and Marco Catani for their kind assistance in the Female AIMS project. I am also very grateful for the generous funding from the Ministry of Education, Taiwan, in supporting my whole doctoral study. I would also like to thank the miscellaneous funds that I have received for conference travel (INSAR, Cultural Division Taipei Representative Office in the UK, Girton College and Cambridge Philosophical Society).

It is the great mentors who have led me into the broad field of psychiatry that set the ground of my research career, especially Drs. Yue-Joe Lee, Shi-Kai Liu, Hao-Wei Wang, Shih-Cheng Liao and Professor Hai-Gwo Hwu. Ever since I entered the field of child psychiatry, I have always been hugely supported by the most prestigious child psychiatrists in Taiwan who foster not only my professional but also personal growth. Without the mentoring, enlightening and persistent encouragements from Professors Wei-Tsuen Soong, Susan Shur-Fen Gau and Dr. Yen-Nan Chiu I would not be able to proceed this far. I would also like to thank Dr. Yu-Yu Wu for her constant concerns to me and sharpening my skills in the ADI-R and ADOS.

My friends from the Girton College and around the UK have accompanied me all the way through. I cannot imagine the Cambridge/British days without you. My most sincere gratitude to all of you – Georg, Maruta, Evelyn, Kazu, Kelvin, Peter, Amr, Po-Hung, Chieh-Ju, Tzi-Huei, Hsiao-Lan, Hsin-En, Yi-Jui and Shu-Sen. And thanks to all my pals in CKMB, who always energise me whenever I am weak.

Lastly, all I have experienced and achieved are meaningless without the company of my beloved families. Without their tremendous unwavering supports and beliefs in me I will never have enough courage to pursue all my goals and overcome the difficulties and challenges. Thank you Mom and Dad for always believing in me and loving me than everything else in your lives. I love you so much. Thank you Ah-Chun for taking care of many things at home for such a long time. Finally, my life is blessed because of you, Pin-Chih. Thank you for always being with me, trusting me and caring about everything of me. My life is for you, now, ever and forever.

Table of Contents

List of Figures

List of Tables

Abstract

A male-predominance in the prevalence of autism spectrum conditions (ASC) has been observed for decades, and has stimulated considerable research interest given its potential relevance to aetiology. However, how biological sex is related to ASC is still poorly understood. One major problem is that ASC in females has often been overlooked in previous studies. This dissertation aims to fill this gap by illustrating the testing between sex and ASC at the behavioural, cognitive and neurobiological levels. Four groups of male and female adults with and without ASC were investigated to test three conceptual models (orthogonal, sex-specific effect, and extreme male brain) describing the relationship between sex and ASC. Particular attention is paid to identify characteristics of females with ASC. In Chapter 2 I show how men and women with ASC are behaviourally both similar and dissimilar. They share comparable levels of autistic symptoms as children yet in adulthood women show fewer autistic features in interactive behaviours, which possibly results from 'camouflage'. In Chapter 3 I show that in the social-emotional domain central to autism, men and women with ASC are both impaired, relative to typical adults. Yet for less central domains (e.g. perceptual attention to detail, motor executive function) there is sex-specific effect, with men with ASC showing impairment compared to controls, an effect not seen in women with ASC. In Chapter 4 I characterise the structural brain features of women with ASC by voxel-based morphometry, and find the result inconsistent with previous reports of men with ASC. In Chapter 5 further morphometric comparisons show women with ASC are different from typical women by a *masculinised* brain morphometry, an effect not seen in males. This constitutes a *sex-specific extreme male brain pattern* and is related to a proxy measure of prenatal

androgen exposure effect. In addition, the morphometric differences between the female groups seem more prominent than those between males. In Chapter 6 the test extends to functional brain imaging. I first provide a proof of method in characterising atypical brain regions in men with ASC by analysing the complexity of intrinsic brain oscillations in terms of fractal geometry. Unlike the distinct group difference between men with and without ASC, women with and without ASC have similar brain oscillation patterns, another example of sex-specific effect. In Chapter 7 I summarise the various sex-ASC relationships at different levels of investigation, and discuss implications from the observed sex differences within ASC (i.e., the sex-specific effects) along with future research directions. In conclusion, this dissertation suggests that although men and women with ASC share similar social-emotional cognitive weakness, and their dispositional traits fit well with the 'extreme male brain' (EMB) pattern, several other behavioural and cognitive features are sexual dimorphic and do not conform to the EMB pattern. Most importantly, their underlying brain characteristics seem to be very different. Women but not men with ASC have structurally masculinised brains. Men but not women with ASC have functionally more random brain oscillations compared to their typical counterparts. We should treat biological sex as a key factor for illuminating the heterogeneity of ASC.

Chapter 1

Introduction:

How is Sex Related to Autism Spectrum Conditions?

1.1 Autism Spectrum Conditions and Heterogeneity

Autism spectrum conditions (ASC) are neurodevelopmental conditions characterised by qualitative impairments in social interaction and communication, alongside the presence of restricted interests, difficulties adapting to change, repetitive and stereotyped behaviours (American Psychiatric Association, 2000; World Health Organization, 1992). The "prototype" comes from two independent case series reported nearly 70 years ago by Leo Kanner (Kanner, 1943) and Hans Asperger (Asperger, 1944). Following a series of early clinical and epidemiological studies (Ritvo, 1977; Rutter, 1968, 1978), it was finally defined as an independent category separated from "childhood psychosis" in the psychiatric taxonomy of the Diagnostic and Statistical Manual of Mental Disorders 3rd edition (DSM-III) (American Psychiatric Association, 1980), characterised by early onset (prior to 30 months old) difficulties in three domains: impaired social development, impaired communicative development and insistence on sameness.

Along with the long-awaited introduction of Asperger's clinical report into the English-speaking world (U. Frith, 1991), the years leading up to the current version of the DSM-IV-TR (American Psychiatric Association, 2000) resulted in increased awareness of the clinical heterogeneity within this diagnostic category (named as "pervasive developmental disorders", PDD) and the introduction of subtypes into the diagnostic framework. These subtypes include autistic disorder, Asperger's disorder, PDD not otherwise specified (NOS), Rett's disorder and childhood disintegrative disorder. This taxonomy is now widely accepted and serves as the basis for daily clinical and educational practice as well as for research. Rett's disorder, however, is

now considered outside the autism regime due to its known genetic/molecular aetiology and distinct course (Monteggia & Kavalali, 2009).

Although the diagnosis of autism was initially conceptualised as being categorical, observations in recent decades have drawn attention to the dimensional nature of autism. Over 30 years ago Lorna Wing had recognised the "autism continuum" as a multi-dimensional condition (Wing, 1975 / 1996; Wing & Gould, 1979). In the past decade, *autistic trait* as a cognitive-behavioural (personality) trait has also been found to be continuously distributed in the general population (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001; Constantino, 2011; Constantino & Todd, 2003). These findings dimensionalise autism into a spectrum from people with few traits, to those with many features. If these result in substantial suffering and functional impairments, this warrants a clinical diagnosis and support. The *dimension within the category of autism* (i.e., multidimensional symptoms in the clinical diagnosis) and *dimension expanding outside the clinical diagnoses* (i.e., autistic traits in the general population) are both well recognised (Baron-Cohen, 2008). This has led to the proposed formal changes of the taxonomy to *autism spectrum disorders* (ASD) in the coming DSM-5 (American Psychiatric Association, 2011), stressing its dual nature, i.e., both categorical and dimensional. With these contexts in mind, the term *autism spectrum conditions* (ASC) used throughout this dissertation overlaps with the diagnostic concept of ASD, corresponding to and inclusive of the DSM-IV-TR diagnoses of autistic disorder, Asperger's disorder and PDD NOS. Particularly for the studies constituting this dissertation, the "ASC group" refers narrowly to those with a clinical diagnosis of either autistic disorder or Asperger's disorder. The usage of ASC is favoured owing to the neutral nomenclature, the less

associated stigma and the stress on the non-disordered aspects (Baron-Cohen, 2000). However, the relevant descriptions and conclusions in this dissertation do not necessarily apply to other's usage of the same term, particularly when it is referring to people who actually do not have a formal clinical diagnosis of ASD.

One major reason to introduce dimensionality onto the stage is the marked individual differences, or *heterogeneity,* of "the autisms" (D. H. Geschwind & Levitt, 2007), that "*the term autism spectrum disorder ... reflects current widespread consensus that autism is best considered as existing on a spectrum with variable manifestations across life span, gender, and intellectual level and/or language ability*" (Happe, 2011). This heterogeneity, not uncommon in human neuropsychiatric conditions, brings about an inevitable dilemma in the real world of research. One strategy to identify ASC characteristics distinct from the (neuro)typical population is by testing as many participants as possible (thereby obtaining a heterogeneous sample), then to contrast them (as a single group) with the typical population, which is also heterogeneous. A classical example of this is in the field of genetics where large sample-sizes are used for genome-wide association studies (GWAS) (Pinto et al., 2010). The downside, however, is the difficulty explaining heterogeneity *within* the spectrum, as well as the huge amount of resources required to conduct this type of large-scale study.

Another strategy is to investigate subpopulations within ASC, such as toddlers (i.e., defined in terms of *age* difference) or people with intact IQ (i.e., defined in terms of *general cognitive ability* difference), or contrasting people with or without developmental language delay. This is a common method used in behavioural, psychological, brain imaging and clinical studies due to practical reasons.

Unfortunately, most studies of this kind are biased by the particular subpopulation examined. For instance, most of the brain imaging studies (especially functional) have focused on people with ASC and without intellectual disability, but very few have directly compared high- versus low-functioning sub-populations (Lotspeich et al., 2004). This results in little knowledge on how the brains are different between high- and low-functioning people. This is likely to reflect the fact that consent as well as cooperation is more easily obtained from the high-functioning subgroup. Besides, most studies have investigated toddlers, children and adolescents; some have investigated young adults, but studies of elderly people with ASC are still very limited (Geurts & Vissers, 2011). This bias towards younger populations is understandable for an early-onset developmental disability but again results in insufficient understanding to ASC across life span. Perhaps the most well studied factor related to heterogeneity is early language delay, which has been extensively studied at the behavioural and cognitive levels (Witwer & Lecavalier, 2008) though less so in brain imaging. These issues and limitations in research contribute substantially to the fragmented and limited understanding of the heterogeneity in ASC.

What is truly surprising is the under-investigation and potentially under-recognition of certain subpopulations. In particular, the differences between males and females with ASC are still poorly understood. Virtually all studies to date have focused exclusively on males or on mixed samples that are predominantly male. For this reason, ASC in the female population is relatively poorly understood. Ignoring the differential features between males and females with ASC may risk losing the opportunity to reduce heterogeneity by sex, and even miss potentially informative indicators about the developmental mechanisms of ASC. On examination

of the history of autism research, however, the relationship between sex and ASC was not completely overlooked in the early years.

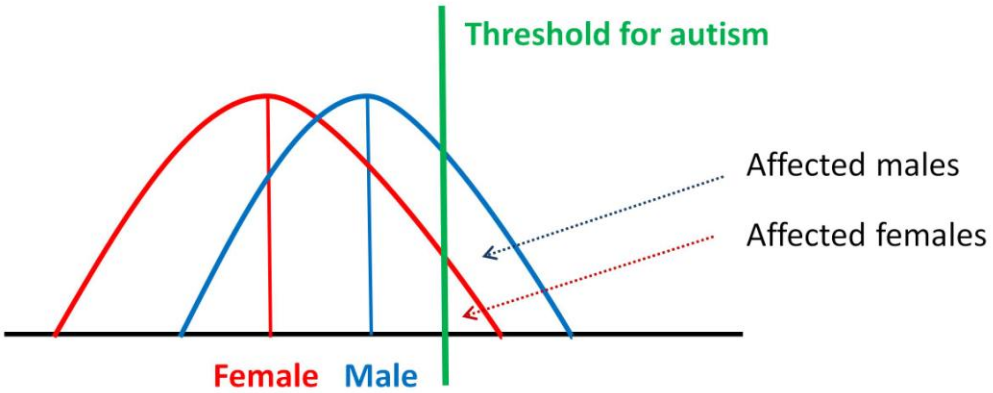
1.2 Why are Autism Researchers Interested in Sex? Early Formulations

ASC is one of the most common neurodevelopmental conditions, affecting approximately 0.6 to 1.57% of the general population (Baird et al., 2006; Baron-Cohen et al., 2009; Newschaffer et al., 2007). Within ASC in general, males outnumber females with a sex ratio of 4.3:1 (Fombonne, 2003, 2005), and this asymmetry in sex ratio has been known from the earliest epidemiological study (Lotter, 1966). Indeed, the initial description of children with "autistic disturbances of affective contact" by Leo Kanner described 8 boys and 3 girls (Kanner, 1943). Similarly, the report on "autistic psychopathy" by Hans Asperger concerned 4 boys and no girls (Asperger, 1944). These were small clinic samples, yet this male bias was also confirmed in the subsequent early epidemiological studies of classic autism with concurrent intellectual disability, where the male:female ratio was 3-4:1 (Lotter, 1966; Ritvo et al., 1971; Rutter & Lockyer, 1967; Wing, 1981; Wing & Gould, 1979). Amongst those with low IQ, the sex ratio decreased to 2:1 (Lord & Schopler, 1985; Wing, 1981) but was nevertheless still present. Despite better recognition of ASC today, these sex ratios and their relation to intellectual ability are consistent with those reported 30 years ago. The sex ratio for individuals with average intelligence is 5.5:1, but 1.95:1 in those with intellectual disability (Fombonne, 2005; Newschaffer, et al., 2007). The first epidemiological study on adults with ASC also confirmed this male predominance by a male versus female odds ratio of 8.62 (Brugha et al., 2011).

This consistent asymmetric sex ratio inspired early researchers' interests into how it might relate to the aetiology of ASC. Observations suggested that girls with classic autism were more severely impaired than boys with classic autism, and in the impaired-IQ population the male:female ratio was lower (Lord & Schopler, 1985; Lord, Schopler, & Revicki, 1982; Tsai & Beisler, 1983; Tsai, Stewart, & August, 1981; Wing, 1981). Tsai et al. (1981, 1983) adopted the *multifactorial liability/threshold model* of disease transmission (Reich, Cloninger, & Guze, 1975) to explain the male predominance by different distribution of genetic liability for males and females. They were viewed as two subforms that had different distributions of liability (which could be *transmissible*, e.g. genetic, or *non-transmissible*, e.g. environmental exposure), and males were generally more liable. It proposed that the reason females were less often affected but once affected were usually more severe was because the liability for females was distributed *away from* being affected compared to that of males. Therefore, higher dose of liability was required for females to be affected; see Figure 1-1 for illustration. This account is elegant in linking the observations in prevalence to a hypothetical liability model. The major weakness is that the liability defined here is treated as reflecting behavioural severity, which now we know is not necessarily the case for a variety of reasons, e.g. differences in genetic penetrance. Furthermore, this model predicts females with autism should have higher rates of affected relatives than males, which was not confirmed by subsequent family genetic studies (Banach et al., 2009; Bolton et al., 1994; Boutin et al., 1997; Goin-Kochel, Abbacchi, & Constantino, 2007; Pickles et al., 2000; Szatmari et al., 2000).

Figure 1-1 The multifactorial liability/threshold model

Males and females are assumed to have different underlying liability to autism. This model predicts that by the same threshold defining autism (green straight line), females (red distribution) need to have higher liability (i.e., more extreme in the distribution) to be affected, and the number of affected females will be smaller than that of males (blue distribution), represented by areas right to the threshold.



Another hypothesis linking asymmetric sex ratio to genetic liability was proposed in Wing's seminal paper (Wing, 1981). She applied the idea of *greater genetic variation in males* (D. C. Taylor & Ounsted, 1972) to explain the sex bias. She proposed that more males might show milder autistic features owing to their wider genetic variability, yet when females had autism it should be contributed by additional types of (brain) pathology. This is a reasonable theoretical formulation given the available evidence during that period. However, the validity of this account should be questioned given the increasing recognition of females with ASC yet without intellectual disability and/or overt brain pathology (Attwood, 2006, 2007; Simone, 2010). The majority of females with ASC do not demonstrate more severe cognitive impairment or additional brain pathology than males. Wing (1981) also referred to

Asperger's initial description of 'autistic psychopathy' as "*an extreme variant of male intelligence*" (Asperger, 1944; U. Frith, 1991), which he referred to the notion of female superiority in "*practical and methodical work*" and male superiority in "*logic ability, abstraction, precise thinking and formulating*". Wing quoted this extreme male cognition idea (but did not take the content forward) with the knowledge in those days of male superiority in visuospatial and mathematical skills and female superiority in language to suggest a *sex-related cognitive superiority and liability model*. This suggested that males were more susceptible to language and communication problems characteristic of autism, but once affected they might be better at developing visuospatial compensation. Females were less vulnerable to autism owing to their innate superiority in language and communication, but if affected they were less likely to develop compensatory visuospatial abilities hence would be profoundly disabled. She nevertheless acknowledged findings that did not fit with this model, for example the equal likelihood for both sexes to have developmental receptive language disorder, and in some children with ASC the better verbal than performance IQ.

From a historical point of view, the *multifactorial liability/threshold model* and the *greater genetic variation in males* notion, both later termed as the 'gender paradox' hypothesis (Eme, 1992), were the first to link sex bias to the aetiology of ASC, particularly of genetic liability. The *sex-related cognitive superiority and liability model*, on the other hand, opened an avenue connecting to the potential cognitive underpinnings. These early formulations led to certain later developments and models, though surprisingly slowly, and remaining understudied.

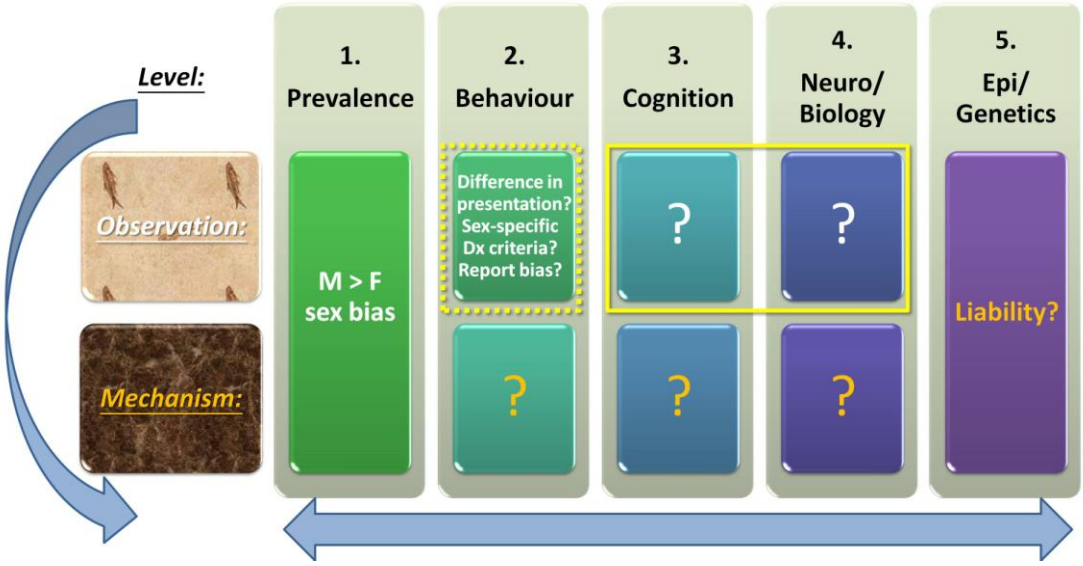
1.3 Redefining Research Questions and Levels of Investigation

The biased sex ratio in the prevalence of ASC may open a door to its aetiology, but it does not pave a shortcut directly to it. One major weakness of the early formulations is that they jump immediately from an *observation* in prevalence to infer a *mechanistic* explanation. Some intermediate links need to be addressed – i.e., descriptions and understandings to the multiple levels lying between prevalence and genetic liability.

The biased sex ratio links sex and ASC together in the research question "*Why is ASC more common in males?*" The ultimate concern, as of the above-mentioned early attempts, is "*How does sex contribute to the aetiology and developmental mechanisms of ASC?*" To approach this question requires intermediate steps, conceptualised in grids in Figure 1-2. On one aspect (y-axis) there are the *observations* on how sex relates to ASC, and the *mechanisms* contributing to these observations. On the other aspect (x-axis) there are levels of behaviour, cognition, neuro/biology and epi/genetics that link aetiology all the way to the observed sex bias in prevalence. Mechanisms cannot be elucidated unless the actual phenomena are clarified (curvy arrow at the left), and final mechanistic understandings require connection and integration among the different levels (double arrow at the bottom). Unfortunately, regarding how sex relates to ASC, up to now we know only little about both the observational and mechanistic aspects at the levels of behaviour, cognition and neuro/biology, and probably a bit more at the epi/genetic level.

Figure 1-2 Research framework for understanding how sex relates to ASC

To illuminate aetiology and developmental mechanisms, one needs to clarify the observations before investigating mechanisms (blue curvy arrow at the left) at different levels, including but not limited to prevalence, behaviour, cognition, neuro/biology and epi/genetics. Integrating mechanistic understandings among these levels will achieve the ultimate illumination (blue double arrow at the bottom). Early studies tried to directly infer liability from observations in prevalence, without considering the intermediate levels. The present dissertation aims to fill this gap by describing the observational aspect of how sex relates to ASC at the levels of behaviour, cognition and neurobiology (yellow rectangles). Dotted rectangle indicates that the present studies only deal with the "soft" problem of the behavioural level; see text below.



It is worth noting that ASC is not the sole neuropsychiatric condition showing a sex bias in prevalence. Conditions with a marked male preponderance usually have an early (childhood) onset and are related to neurodevelopment, for example autism, developmental language disorders, attention deficit/hyperactivity disorder, dyslexia and conduct disorder. On the contrary, conditions with a marked female preponderance often have a late (adolescence) onset and are associated with emotion regulation, for example depressive/mood disorders, anxiety disorders and eating

disorders (Rutter, Caspi, & Moffitt, 2003; Zahn-Waxler, Shirtcliff, & Marceau, 2008). This unique pattern of sex difference in psychopathology points to an avenue to study the causal mechanisms (Rutter, et al., 2003). To elucidate sex-ASC relationship by starting with the observational level fits well with this research direction in developmental psychopathology.

In the past three decades a few studies have tried to illuminate the relationship between sex and ASC, contributing to a better understanding to the heterogeneity of ASC. The key findings at each level are reviewed in the following sections.

1.3.1 Prevalence

As described earlier, the male bias in people diagnosed with ASC has been constantly reported. The sex ratio is lower in impaired-IQ populations and higher in high-functioning groups. Whether this reflects the actual sex ratio is still under debate, since high-functioning females with ASC might be under-recognised due to their 'non-male-typical' presentation or ability to camouflage their difficulties (Attwood, 2006, 2007; Baron-Cohen et al., 2011; Gillberg, 2002; Gillberg & Coleman, 2000; Kopp & Gillberg, 1992, 2011). Thus, studies comparing the behaviour of males and females with ASC are required to inform further prevalence research.

In the general population males on average exhibit more 'autistic traits' than females, measured by questionnaires such as the Quantitative Checklist for Autism in Toddlers (Q-CHAT) (Allison et al., 2008), the Autism Spectrum Quotient (AQ) child (Auyeung, Baron-Cohen, Wheelwright, & Allison, 2008), adolescent (Baron-Cohen, Hoekstra, Knickmeyer, & Wheelwright, 2006) and adult versions (Baron-Cohen, Wheelwright, Skinner, et al., 2001), the Social Responsiveness Scale (SRS)

(Constantino & Todd, 2003), the Autism Spectrum Screening Questionnaire (ASSQ) (Posserud, Lundervold, & Gillberg, 2006), and the Childhood Autism Spectrum Test (CAST) (Ronald et al., 2006; Ronald, Happe, Price, Baron-Cohen, & Plomin, 2006; J. G. Williams et al., 2008). These questionnaires probe the behavioural and cognitive features of ASC (both typical and attenuated) and/or characteristics commonly presented in relatives of people with ASC. This sex difference in distribution in the general population goes along with the male predominance in the clinical population. It suggests that for above-average level of autistic traits males outnumber females.

1.3.2 Behaviour

The behavioural sex differences have been described in clinician's experiences, case studies and observations in research. For example it has been reported that boys with ASC have greater unusual visual responses and motor stereotypy, and less appropriate play (Lord, et al., 1982; Volkmar, Bregman, Cohen, & Cicchetti, 1988). Girls with ASC have been reported to have better superficial social communication skills (Attwood, 2006, 2007; Gillberg & Coleman, 2000) and fewer peculiar narrow interests (Gillberg & Coleman, 2000; Kopp & Gillberg, 1992; Wolff & McGuire, 1995). Nevertheless how differences in cognitive and developmental levels affect these observations is still not clear.

Up to now only eight studies (including a published paper from this dissertation) focused on comparing age- and IQ- (if appropriate) matched males and females with ASC on their core autistic behaviours assessed by standardised instruments (Carter et al., 2007; Hartley & Sikora, 2009; Holtmann, Bolte, & Poustka, 2007; Lai et al., 2011; McLennan, Lord, & Schopler, 1993; Pilowsky, Yirmiya, Shulman, & Dover, 1998;

Solomon, Miller, Taylor, Hinshaw, & Carter, 2011; Tsai & Beisler, 1983), which will be reviewed in detail in Chapter 2. In short, there is inconsistency in results. Some reported no sex differences (Tsai, Pilowsky, Holtmann, Solomon), while others did (McLennan, Carter, Hartley, Lai). These studies investigated people with ASC in different age and IQ ranges, highlighting the heterogeneity again. How males and females with ASC are similar and/or dissimilar to each other may be dependent on which subpopulation they belong to. They may also have differential developmental trajectories in regards to behavioural changes.

There is an even more complicated issue here. The potential sex difference in behavioural presentation leads to the suspicion that females may be mis- or under-diagnosed because the current criteria are 'male-biased' (Baron-Cohen, et al., 2011; Constantino, 2011; Kopp & Gillberg, 1992, 2011; Wing, Gould, & Gillberg, 2011). This brings up the question whether there should be sex-specific diagnostic criteria (Skuse, 2007). If this is to happen, it will first require a new 'core' definition of ASC using features other than behavioural (e.g. cognitive or neurobiological), so that behaviours of males and females can be fairly compared. I call this the '*hard*' problem due to the fact that ASC is currently defined by behaviour. Whatever cognitive or neurobiological features found to be core to ASC are about the ASC defined by the current 'male-typical' behavioural criteria. It is only after we have accumulated enough cognitive and neurobiological observations *for both sexes*, and have reached consensus on defining ASC by behaviour *and* other levels, that this 'hard' question can be addressed properly.

On the other hand, what was adopted by almost all of the previous studies could be viewed as solving the '*soft*' problem. We are still able to gain insights into sex

differences in ASC in a range of behavioural presentation by comparing males and females whose ASC is defined by a consensus criteria (e.g. DSM-IV). This is a rather conservative approach because only those who have the core behavioural features defined by the criteria are compared. It is however a safe place to start exploring sex-ASC relationship since it provides a common basis for the definition of ASC for males and females. The studies in this dissertation follow this approach.

The last issue at the behavioural level is the potential care-giver report bias. The diagnosis of ASC relies significantly on early developmental history. If care-givers are biased by own gender stereotype or expectation when observing or interpreting the child's behaviour, their reports will be biased (Holtmann, et al., 2007). However the bias could go either way. They may expect more socially desirable behaviours from girls than boys, and indeed the perceived discrepancy between actual and ideal behaviours is often larger for females than males (Crick & Zahn-Waxler, 2003). This would result in an over-report of atypical behaviours for girls and an under-report for boys. On the other hand, the same biased expectation or stereotype may modify the care-givers' interpretation of children's atypical behaviours, for example lack of social interest interpreted as 'shy' for girls (Attwood, 2006; Ernsperger & Wendel, 2007) thus viewed as natural and overlooked. This would result in an under-report of atypical behaviours for girls. Direct comparisons between objective observations to care-giver reports on children's behaviours are needed to elucidate this issue.

1.3.3 Cognition

Over the decades researchers have comprehensively characterised the cognitive features of ASC and have generated a range of cognitive theories for ASC in different

domains (summarised in Chapter 3). However, few of them incorporated sex and put it at centre stage. Furthermore, cognitive sex differences in ASC are rarely investigated. The few conceptualisations that relate sex to the characteristics and emergence of ASC includes the *extreme male brain (EMB) theory of autism*, first anecdotally suggested by Hans Asperger in his initial case reports (Asperger, 1944), briefly mentioned by Lorna Wing (Wing, 1981) and finally formally proposed by Simon Baron-Cohen (Baron-Cohen, 2002). The *sex-related cognitive superiority and liability model* described earlier (Wing, 1981) is another example, which shares similar basis (i.e., the observations of sex differences in cognition) with the EMB theory.

The EMB theory is based on its twin theory describing sex-dimorphic cognitive features in the general population, the *empathizing-systemizing theory* (Baron-Cohen, 2002). *Empathizing* is defined as the drive to identify another's emotions and thoughts and to respond to these with an appropriate emotional reaction (Baron-Cohen, 2003). This reflects the two components of empathy: *cognitive* (also called 'mentalizing' or 'theory of mind') and *affective*. Empathizing is a particularly powerful cognitive tool for understanding *agentive* events and interactions, i.e., for making sense of the complex and frequently unpredictable social world. *Systemizing* is defined as the drive to analyse and construct *rule-based* systems (Baron-Cohen, Richler, Bisarya, Gurunathan, & Wheelwright, 2003; Wheelwright et al., 2006). It involves identifying the rules that govern the system (input-operation-output rules) to predict how it will behave. Systems may be mechanical (e.g. the mechanics of objects), natural (e.g. pattern of tidal waves), abstract (e.g. the syntax of language) or even social (e.g. a management hierarchy in industry). Systemizing is a particularly powerful cognitive

tool for understanding the *non-agentive* aspects of our surrounding environment.

The axes of empathizing and systemizing capture differences in cognitive styles between males and females (Goldenfeld, Baron-Cohen, & Wheelwright, 2005). In addition, individuals with ASC are over-represented at opposite extremes of both axes: hyper-systemizing and hypo-empathizing (Baron-Cohen, Knickmeyer, & Belmonte, 2005). Using well-validated psychometric instruments, the Empathy Quotient (EQ) (Baron-Cohen & Wheelwright, 2004) and the Systemizing Quotient (SQ) (Baron-Cohen, et al., 2003), females on average have a higher level of empathy relative to their systemizing (a profile termed Type E) whereas males on average have the opposite profile (Type S). Individuals with ASC show below average empathy alongside intact or even superior systemizing (Extreme Type S) (Baron-Cohen, 2002).

Therefore, the EMB theory suggests that ASC is a hyper-masculinised presentation in terms of aspects of cognition that show a sex difference in the general population, specifically in the domains of empathizing and systemizing (Baron-Cohen, 2010). Nevertheless, whether the cognitive and behavioural features of ASC are presented differently in affected males and females is not explicitly predicted or described in the theory. Questionnaire-based studies suggest there may be no difference, that typical sex differences in autistic traits, empathy and systemizing in adults are absent in ASC (Baron-Cohen, Wheelwright, Skinner, et al., 2001; Wheelwright, et al., 2006). This evidence extends to parent-reported autistic characteristics in childhood (Auyeung, et al., 2008) and in adolescence (Baron-Cohen, Hoekstra, et al., 2006). Similarly, no sex differences within ASC are seen on the child versions of the Empathy Quotient and Systemizing Quotient (Auyeung, Wheelwright, et al., 2009), the Childhood Autism Spectrum Test (J. G. Williams, et al., 2008) and

the Quantitative Checklist for Autism in Toddlers (Allison, et al., 2008). Given that in the general population males score higher than females on all of these instruments, the absence of a sex difference in autism is consistent with the view that females with ASC show a masculinised profile.

On the other hand, there are a few other studies beginning to test if males and females with ASC have different cognitive features, such as distinct developmental and cognitive profiles (Carter, et al., 2007; Koyama, Kamio, Inada, & Kurita, 2009) and executive function (Bolte, Duketis, Poustka, & Holtmann, 2011; Lemon, Gargaro, Enticott, & Rinehart, 2011). The results from these initial studies indicate that the two sexes are partially different from each other. This suggests plausible *sex-specific cognitive profiles* in ASC. More studies are needed for validation and further exploration.

1.3.4 Neuro/Biology

Over the past decade some influential neurobiological theories for autism have been proposed, e.g. the complex information processing deficit (Minshew, Webb, Williams, & Dawson, 2006) and the dysconnectivity theory (Belmonte et al., 2004; Just, Cherkassky, Keller, & Minshew, 2004). However, few of them tackle how sex relates to ASC. In early years it was proposed that in ASC the language deficits were due to left hemisphere damage and preserved perceptual skills due to intact right hemisphere function (Lord, et al., 1982). Given the observation of less lateralisation in females, they were more resistant to left hemisphere damage (which was held to cause ASC in males) thus less likely to be affected by ASC. If females got ASC they would need more extensive bilateral brain damage to produce symptoms such as language

deficit. This model is less favoured nowadays due to the lack of evidence showing restricted left hemisphere damage in males with ASC. It also fails to explain the core social deficits in ASC since no evidence confirms that social cognitive processing in the brain is restricted to the left hemisphere.

Recently there is increasing evidence suggesting potential sex-specific neurobiological and biological features for ASC. By identifying the growth trajectory of brain volume, *early brain overgrowth* starting before the age of one has been proposed as a cardinal feature for ASC (Courchesne, Carper, & Akshoomoff, 2003; Courchesne & Pierce, 2005; Courchesne et al., 2007). Female toddlers with ASC showed greater volumetric deviance from typical girls compared to that of boys at temporal grey matter (even larger in girls with ASC) and cerebellum (larger in boys but smaller in girls with ASC) (Bloss & Courchesne, 2007), as well as in the amygdala (even larger in girls with ASC) (Schumann, Barnes, Lord, & Courchesne, 2009). The overgrowth trajectory was also more obvious and involved more brain structures in girls compared to boys (Schumann et al., 2010). Until now there have been no studies exploring sex differences in neuroanatomical features in older children, adolescents or adults with ASC as to how these are different from their typical counterparts respectively.

The other evidence came from the observation in serum biomarkers (Schwarz et al., 2010). It was found that serum biomarkers in male adults with ASC included 24 analytes, among them mainly cytokines and inflammatory molecules. For female adults with ASC the biomarkers were 17 analytes consisted of mainly androgen-related, growth hormone and insulin-related molecules. In multivariate statistical classification, this male "fingerprint" successfully separated male adults

with ASC from typical men, but it failed to do so for the female groups. Similarly, the female "fingerprint" well classified diagnostic group membership in females but failed in males. This sex-specificity, although observational, also provides implications to potential sex-specific mechanisms for the emergence of ASC.

1.3.5 Epi/Genetics

Of all levels, perhaps most attention focusing on the link between sex and ASC is at the genetic level. As was the intention of the early models described previously, most researchers are willing to interpret the sex bias in the prevalence to a sex difference in the liability to ASC, and genetic factors are naturally thought to contribute most to this liability. The overarching assumption is that *females are less vulnerable* to developing ASC.

Genetic heterogeneity in ASC is large. This is not due to single genes (apart from genetic syndromes with autistic presentation, e.g. fragile X syndrome, Rett's syndrome, tuberous sclerosis) since multiple genes are involved and interact (Abrahams & Geschwind, 2008; Voineagu et al., 2011). Therefore, the sex bias in ASC is difficult to explain by X-linked or other traditional inheritance models (Schanen, 2006). Several models further propose that *X chromosome epigenetics* may be responsible (Beaudet, 2007; Rivet & Matson, 2011). The first is the *imprinted-X liability threshold model* which suggests a protective effect from the paternally inherited X chromosome (Skuse, 2000), inspired from the findings in girls with Turner syndrome (TS) who have only one X chromosome. Girls with TS whose X chromosome was inherited from the father showed better social communication skills than those whose X inherited from the mother. Skuse hypothesised that certain genes

expressed only on the paternal X, by genomic imprinting, act as protective factors against the social communication difficulties in TS, and thus by extrapolation, may also protect against ASC. The less vulnerability in females could then be explained by the fact that males can only have maternal X chromosome, whereas females have both maternal X and the protective paternal X. However, specific genes have not been identified yet to support this model.

The second model, the *X-inactivation/X-linkage hypothesis*, suggests there are quantitative trait loci (QTL) located on the X chromosome responsible for social cognitive traits related to autism, which may then relate to the sex bias in ASC (Loat, Asbury, Galsworthy, Plomin, & Craig, 2004; Loat, Haworth, Plomin, & Craig, 2008). The model predicts that due to *skewed X-inactivation* (i.e., for the two X chromosomes in females, the inactivation of one does not occur randomly), if there are "autistic trait QTL" on the X chromosome, there will be differential expression of these traits such that monozygotic female twins will be less similar to each other than monozygotic male twins, whereas dizygotic female twins will be more similar to each other than dizygotic male twins. These predictions were confirmed in a large typical population twin sample aged 7 to 8 (Loat, et al., 2008). However this theory only implicates the presence of autism-related QTL on the X chromosome, but does not explain exactly how it contributes to the sex bias in prevalence.

The third model, *X-linked male extremes*, is proposed based on the finding that X chromosome contains a high density of genes important for cognitive abilities (Zechner et al., 2001). It is suggested that males have more extreme X-linked phenotypes than females because in females X-linked gene expression is averaged out by X-inactivation across cells, so it is less likely for them to develop an extreme

X-linked phenotype, including ASC (Skuse, 2005, 2006).

There are another two hypotheses not focusing on the X chromosome. The first, different from the vulnerability perspectives, suggests that males and females are equally at risk for ASC in terms of genetic predisposition, but other factors (genetic, biological or experiential) enable females to better *compensate* for these risks (Skuse, 2007). Second, based on findings of different copy number variations in multiplex versus simplex autism (Jacquemont et al., 2006; Sebat et al., 2007), it was proposed that ASC could be classified as *sporadic* (having mainly *de novo* mutations occurring first in the parental gametes) or *inherited* (having mainly inherited mutations). For still unknown reason females are more resistant to display the effect from the *de novo* mutations on the autosomes (i.e. *reduced autosomal penetrance*), hence less likely to develop the full set of ASC symptoms (Beaudet, 2007; Zhao et al., 2007). These two hypotheses require further tests for clarification.

1.3.6 Scope, focus and designs of this dissertation

At all of these levels, extensive work is still required to facilitate understanding of how sex relates to ASC. The first aim of this dissertation is to provide a multi-level characterisation of female adults with ASC, which has never been systematically studied before. The second aim is to further explore the relationship between sex and ASC at the behavioural, cognitive (Chapters 2 and 3) and neurobiological (Chapters 4, 5 and 6) levels (Figure 1-2, yellow rectangles). These will potentially fill the gaps in the current literature. Biological, genetic and epigenetic aspects will not be investigated here, but will be important avenues to pursue in future studies for cross-level integration.

The present study designs are all based on group comparisons, either in a pair-wise fashion or by a two-way factorial design. Since this dissertation is the first to explore how sex is relevant to ASC, a 'polarised' sample selection provides greater power to characterise the relationships. That is, the ASC group consisted of those with a clinical diagnosis and confirmation of symptom severity by a researcher, and the typical group included those without a clinical diagnosis of ASC *and* no family history of ASC. This is not contrary to the dimensional perspective. Instead we hope the present initial investigation with its simple but powerful statistical design may be able to provide clues to how the dimensional nature of ASC and its heterogeneity can be further clarified.

An observational understanding not only provides a way to characterise a condition, but also serves as the basis to generate hypotheses about its emergence. Although the aim of this dissertation is to clarify how sex and ASC interacts at the observational level, what is discovered and described will help generate new hypotheses to test relevant underlying mechanisms (Figure 1-2, orange question marks). However, an intrinsic limitation to any cross-sectional design is that what is found is simply correlational. Other study designs, such as an experimental intervention design or a longitudinal cohort study, will provide more valid tests exploring the mechanistic aspects.

1.4 Sex, Gender, Cognition and the Brain

Since one major factor to be investigated is sex, it is helpful to provide a relevant overview. Issues surrounding sex, gender, sex differences and similarities are perhaps one of the most debated topics in the fields of psychology and neuroscience, and a

detailed discussion is beyond the scope of this dissertation. Only the key concepts and most consistent findings relevant to the present studies will be summarised below.

1.4.1 Defining sex and gender

In the field of psychology and behavioural sciences, the term *sex* is conventionally associated with the *biological* (and specifically chromosomal) difference between males and females (i.e., XY vs. XX). The term *gender* refers to the *psychological* characteristics associated with males and females (i.e., masculine vs. feminine) in a particular cultural system, and is partly socially constructed (Unger, 1979). In the field of social sciences and cultural studies, gender is sometimes even more broadly viewed as a social structure, defined as "*the structure of social relations that centres on the reproductive arena, and the set of practices that bring reproductive distinctions between bodies into social processes*" (Connell, 2009). The constructs referred to by these terms are not always tightly associated with each other (e.g., some biological females will have masculine traits). Therefore they should not be considered as synonyms (Hamilton, 2008). In this dissertation the term *'sex'* will be used rather than *'gender'* since the biologically defined distinction is of current interest. Gender is too broad a construct to be referred to and investigated here. However, any biological process is in constant dynamic interaction with the (social) environment and is modified by experiences. The focus on *sex* does not indicate an essentialist stance. Rather it is used as a descriptive classification. It is acknowledged that both nature and nurture play roles in what is observed and found.

It is also important to note that although *sex* will be treated simply as binary (i.e., male and female), the fact is not so simple. Biological sex itself is multi-level,

spanning from chromosomal, hormonal, anatomical to physiological aspects. Each aspect is in fact not absolutely binary but is rather distributed in a bimodal fashion. Even for the fundamental chromosomal sex there exist a range of intermediate conditions (e.g. XXY). The analytical framework applied here treats sex as binary for the same reason as we treat ASC in a binary fashion, in the hope that exploring the two typical subpopulations in the bimodal distribution will provide the greatest power to reveal differences. However this strategy cannot describe individual differences within each binary category. Therefore, findings should be treated as indicating the average for each sex, not that all people of one sex conforming to it.

1.4.2 Sex differences and similarities in behaviour and cognition

Many studies have investigated aspects of human behaviour and cognition to identify if there is a consistent pattern of sex differences and similarities (Halpern, 2000; Hamilton, 2008; Kimura, 1999). Although some are frequently reported to show a sex difference, for example the male advantage in dynamic visuomotor tasks (Hamilton, 2008) or the female advantage in empathy (Baron-Cohen, 2003), many of the remaining differences depend on the nature of the task. There is no simple pattern of sex difference in broad domains, for instance the falsely perceived notion that males are better in mathematics and females better in language. A summary on 46 meta-analyses (J. S. Hyde, 2005) actually shows that out of 124 reported effect sizes in behavioural and cognitive sex differences, 30% are close to zero (Cohen's $d \leq 0.1$) and 48% are small ($0.11 < d < 0.35$). This led to the 'gender similarities hypothesis' (J. S. Hyde, 2005, 2007) stressing more sex similarities than differences in human cognition and behaviour. The exceptions include motor performance, which is usually

better in males, and some measures of sexuality, which show higher levels of activity in males. Males also show more aggression and better mental rotation ability at a moderate magnitude (Hines, 2010).

Many sex differences also show a developmental trend, for example young girls perform comparably to boys on mathematics but boys on average score higher after teenage (J. S. Hyde, Fennema, & Lamon, 1990). One of the most consistently observed sex differences is in the *childhood* choice of toys, playmates and games (Hines, 2010). Furthermore, sex differences in performance are also highly dependent on the context of the task. In sum, although there are a few exceptions (e.g. motor function, visuomotor processing, tendency to aggression and sexual activity [all larger in males] vs. empathy [possibly larger in females]), most sex differences vary in magnitude at different ages and are dependent on the context in which the task is performed (J. S. Hyde, 2005). The key conclusion is that *within-sex variability outweighs between-sex variability*.

1.4.3 Sex difference in the brain

A large number of studies have investigated the anatomical aspects of neural sex differences, including size and shape features. From cross-sectional studies of adults the most consistent finding is that males have larger brain size than females (Cosgrove, Mazure, & Staley, 2007; Goldstein et al., 2001), an effect which is not fully explained by their larger body size (Ankney, 1992). For localised brain structures, the largest voxel-based morphometry (VBM) study to date shows that males have proportionally (accounting for total grey matter volume) larger bilateral amygdala, left temporal pole and cerebellum superior lobule, whereas females have

larger orbitofrontal and cingulate cortices, inferior frontal gyri, inferior parietal lobule and perisylvian language regions (i.e., planum temporale and Heschl's gyri) (Good et al., 2001). Most of these regions have been confirmed by other VBM studies (X. Chen, Sachdev, Wen, & Anstey, 2007; Cheng et al., 2009; Wilke, Krageloh-Mann, & Holland, 2007; Yamasue et al., 2008), region of interest (ROI) studies (Goldstein, et al., 2001; Koscik, O'Leary, Moser, Andreasen, & Nopoulos, 2009; Nopoulos, Flaum, O'Leary, & Andreasen, 2000; Schlaepfer et al., 1995; Tiemeier et al., 2010) and study on grey matter concentration (Luders et al., 2005) which additionally reveals other regions such as larger caudate, pre- and postcentral gyri in females. Furthermore, comparing brain size-matched male and female adults reveals that females have larger caudate, left superior frontal and temporal gyri than males, indicating the effect of total brain size on the observed local volumetric sex differences is negligible (Luders, Gaser, Narr, & Toga, 2009).

Studies on the geometric aspects of brain anatomy also reveal sexual dimorphism. Women have significantly thicker cortices than men, especially in frontal, parietal and occipital cortices (Im et al., 2006; Luders, Narr, et al., 2006; Sowell et al., 2007). Larger cortical surface area and increased gyrification have also been reported for females than males (Luders, Thompson, et al., 2006; Nopoulos, et al., 2000). For brain asymmetry, the left-ward asymmetry of perisylvian language regions (N. Geschwind & Levitsky, 1968) is more pronounced in males (Good, et al., 2001; Wada, Clarke, & Hamm, 1975; Witelson & Kigar, 1992).

The growth of the brain is also sexually dimorphic. After controlling for birth weight, male babies have larger cortical grey matter (10% larger), white matter (6% larger) and subcortical structure (7% larger) volumes than females (Gilmore et al.,

2007). From childhood onwards, white matter grows linearly in both sexes but the trajectory is steeper in males, particularly during adolescence (Giedd et al., 1999; Lenroot et al., 2007). Grey matter maturation follows a nonlinear (inverted-U) fashion (Shaw et al., 2008) and peaks at early adolescence in a lobe-specific manner (except occipital lobes) with males peaking later than females (Giedd, et al., 1999; Lenroot, et al., 2007). The cerebellum also grows in an inverted-U fashion peaking in adolescence, again with males later than females (Tiemeier, et al., 2010).

Studies have also investigated the functional aspect of the brain. The only study to date demonstrating sex differences in resting-state brain activity shows that females have stronger connectivity within the default network (by both seed-based and independent component analyses) and higher power of the low-frequency spontaneous oscillations locally at the posterior cingulate cortices (Biswal et al., 2010). On performing advanced mentalizing tasks, typical males show less activity in the bilateral inferior frontal gyri (BA 44/45) compared to typical females (Baron-Cohen et al., 2006). In addition, women recruit more mirror neuron areas than men in empathic face-to-face interaction (Schulte-Ruther, Markowitsch, Shah, Fink, & Piefke, 2008). They also show stronger activation in the medial prefrontal cortex in anticipating and experiencing pain than men, indicating higher self-related attention (Straube, Schmidt, Weiss, Mentzel, & Miltner, 2009). There are also other reports of sex differences in the recruitment of brain regions on perceiving facial emotion (Aleman & Swart, 2008), judging facial attractiveness (Cloutier, Heatherton, Whalen, & Kelley, 2008), mentalizing (Krach et al., 2009), auditory gating (Tomasi, Chang, Caparelli, & Ernst, 2008) and basic visuospatial processing (Clements-Stephens, Rimrodt, & Cutting, 2009).

1.5 How is Sex Related to ASC? The Different Models

The omnibus way to clarify how sex relates to ASC is to identify the statistical relationship between them. Since both sex and ASC are defined as a factor with two levels (sex: male or female; ASC diagnosis: with or without ASC), their relationship can be understood under the framework of a two-way factorial analysis of variance (ANOVA). This provides the test for two competing models with regards to the interaction between sex and ASC, i.e., either they have a statistical interaction or they do not. In this dissertation the latter is called the *Orthogonal* (OG) model and the former the *Sex-Specific Effect* (SSE) model. A third possibility, the *Extreme Male Brain* (EMB) model, stems from the cognitive EMB theory and tests a very specific type of sex-ASC relationship. The following sections illustrate the basic concepts for each model. How they are applied to the tests at the brain level will be further elaborated in Chapter 5.

1.5.1 Model 1: Orthogonal (OG)

At the univariate level, for an outcome variable which could be a cognitive (e.g. language performance) or brain measure (e.g. a voxel value), if sex and ASC have independent effects on this variable (which do not need to be statistically significant main effects) then they are *orthogonal* (see Figure 1-3 left panel for conceptual illustration). The key is that there is *no statistical interaction* between the two factors. This means that the effect of ASC on the outcome measure is not influenced by the sex of the person, and the effect of sex on the measure is not influenced by whether this person has ASC or not.

An example of an OG relationship can be illustrated as follows. Males, disregarding having ASC or not, are on average taller than females (so there is a main effect of sex on height). People with ASC, disregarding sex, are about the same height as typical controls (so there is no main effect of ASC on height). Most importantly, how sex influences height does not differ between ASC and typical people (so there is no sex-by-ASC interaction). In other words, the diagnosis does not affect how sex has an effect on the person's height. This example illustrates that sex and ASC have *independent* effects on height.

1.5.2 Model 2: Sex-specific effect (SSE)

For an outcome variable, if sex affects how ASC has an effect (or vice versa), then there is an *interaction* between the two factors. In other words, how ASC affects the measure of interest is different in males versus in females (i.e., dependent on sex), or that how sex affects the measure of interest is different in people with versus without ASC (i.e., dependent on ASC diagnosis). This is generally referred to as a *sex-specific effect* (Figure 1-3 middle panel).

For example, males, disregarding having ASC or not, are on average taller than females (so there is a main effect of sex on height). People with ASC, disregarding sex, are about the same height as typical controls (so there is no main effect of ASC on height). However, in this example the influence of sex on height differs between ASC and typical groups. Males with ASC are on average 20 cm taller than females with ASC, whereas typical males are on average 5 cm taller than typical females. In a two-way factorial ANOVA analysis, there is a significant *sex-by-ASC interaction*, indicating that the effect of sex on height is larger in ASC than in typical people. In

this example the effect of sex on height is dependent on the diagnosis.

1.5.3 Model 3: The extreme male brain pattern (EMB)

The EMB theory at the cognitive level provides an additional explanation of the sex-ASC relationship that can also be tested. However, it is not directly detectable by the two-way factorial ANOVA framework. The EMB pattern indicates that for an outcome variable, the effects of sex and ASC follow the same direction implied by the EMB theory (Figure 1-3 right panel), i.e., male > female *and* ASC > typical (or male < female *and* ASC < typical). Therefore, the EMB model is different at the basic framework level from the SSE and OG models, that it is actually a *1-dimension model*, in contrast to the 2-dimension nature (i.e., sex and ASC) of the other two models.

Using the same example, the EMB model has three requisites:

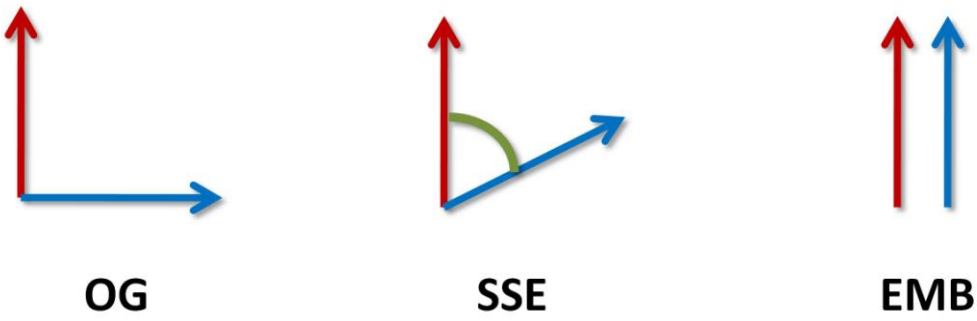
1. There is a typical sexual dimorphism (e.g., typical males are taller than typical females).
2. Males with ASC are more *masculinised* compared to typical males (e.g., males with ASC are even taller than typical males).
3. Females with ASC, though not explicitly described in the original theory, should be similar to males with ASC, hence are also more *masculinised* compared to typical females (e.g., females with ASC are taller than typical females).

Owing to this 1-dimension nature (i.e., sex and ASC converge as one dimension), it *cannot* be fully tested under the framework of a two-way factorial ANOVA. The only proper way to test if the EMB model fits is to examine whether the differences between groups match the three requisites of pair-wise relationship outlined above. However if the sex-ASC relationship does conform to the EMB model predictions,

using a two-way factorial ANOVA may either show (i) a combination of main effects of sex and ASC but no interaction; (ii) a combination of main effects of sex and ASC but with a significant *ordinal* interaction, in which case the main effect(s) are interpretable; or (iii) the groups lined in the order as predicted by the EMB model, but no main effect or interaction effect exists. These complicated possibilities once again demonstrate that a two-way factorial ANOVA framework is *not* adequate to test the EMB model.

Figure 1-3 Models for the relationships between sex and ASC

The geometric relationship between the arrows illustrates the three models on the relationship between sex (red arrow) and ASC (blue arrow). An 'orthogonal' (OG) model (left) indicates the two effects are independent of each other. A 'sex-specific effect' (SSE) model (middle) indicates the effect of one is dependent on the other, so there is an interaction between them (represented by the green curve indicating a non-orthogonal angle). An 'extreme male brain' (EMB) model (right) indicates the two effects are on the same direction, i.e., sex and ASC show the same effect. This actually merges the two dimensions (sex and ASC) into one.



1.6 Which Subpopulation to Study? Reasons, Strengths and Limitations

There is one last issue to be addressed regarding the research design of this dissertation. For the group with ASC, adults (aged over 18) with average or above-average IQ and without major cormorbid conditions[1] were recruited. There are reasons for this, as well as pros and cons.

Owing to the early onset and major impacts of ASC on the development and learning of the child, as well as the quality of life of the whole family, much attention has been paid to early detection, intervention and support. There is no doubt that research in this direction should continue since early detection and intervention indeed have brought substantial improvements to the child's learning and functioning (Vismara & Rogers, 2010; Zwaigenbaum, 2010). However, ASC persists across the life span. It is equally important to pay attention to adults, whose presentation, development and needs are invariably different to those at younger ages. In particular there are many people on the spectrum who have not yet been formally diagnosed with ASC, despite their long-standing suffering in social communication difficulties and behavioural emotional problems (Tantam, 2000). There is now increasing attention to improve the awareness, assessment, diagnosis, services and supports for adults with ASC (Department of Health, 2010; Murphy, Beecham, Craig, & Ecker,

[1] These excluded comorbid conditions include medical conditions such as epilepsy and genetic syndromes commonly associated with autism presentation (e.g. fragile X syndrome, tuberous sclerosis complex), as well as certain major psychiatric conditions (e.g. hyperkinetic disorder, Tourette's syndrome, psychotic disorders, substance-use disorders).

2011; Tantam, 2003). For example, the first epidemiological study in the general population for adults with ASC has just published this year, showing a prevalence of 9.8 per 1000 in England (Brugha, et al., 2011). Most importantly these adults with ASC identified in the community are socially disadvantaged and tend to be unrecognised. This is one of the main reasons that this dissertation focuses on the adult population, hoping to achieve a better understanding to adults with ASC.

We also focus only on those without common major comorbid conditions. The main strength of this strategy is that our sample will be a relatively homogenous subpopulation, potentially 'purer' ASC. As pointed out at the beginning of this chapter, research focusing on a more homogenous subpopulation will be advantageous as it enables greater power to detect differences from the typical population. This is indeed the strategy we opt for, trying to solve just one piece of the puzzle from the whole jigsaw of ASC.

Pros are always accompanied by cons. Owing to this restricted sample selection, the results cannot be generalised to the other subpopulations of ASC. Furthermore, for adults with ASC and with average or above-average IQ, the majority of them were not diagnosed until late childhood, adolescence or even adulthood. Whether there are fundamental differences between these late-diagnosed people and the early-identified group is an open question. Lastly, as a cross-sectional study for adults, all observations are snapshots of their current condition, and all inferences are correlational rather than causal. What is observed in adulthood is inevitably a mixture of primary characteristics and secondary (including compensatory) changes, which cannot be disentangled by the present design. Given the marked heterogeneity of ASC, it is important to bear these limitations in mind when interpreting the results reported

by this dissertation.

1.7 Dissertation Plan

Chapter 2 examines the behavioural similarities and differences between male and female adults with ASC. Chapter 3 extends the comparison to include typical groups under a two-way factorial design, on a broader range of cognitive tasks clustered by the major cognitive domains related to ASC. Chapter 4 characterises the neuroanatomical features of female adults with ASC in terms of brain morphometry, which is seldom studied in the past. Chapter 5 further examines how sex and ASC diagnosis interact in terms of brain morphometry. Chapter 6 first demonstrates a proof of method in characterising resting brain functional organisation properties, then examines how sex and ASC diagnosis interact at the level of resting brain activity. Chapter 7 provides a summary and integration of the findings on sex-ASC relationships at the different levels and discusses implications for future investigations into the developmental mechanisms of ASC.

Chapter 2

Behavioural Comparison of Male and Female Adults with High Functioning Autism Spectrum Conditions

2.1 Introduction

Autism spectrum conditions (ASC) affect more males than females in the general population. However, within ASC it is unclear if there are phenotypic sex differences. Testing for similarities and differences between the sexes is important not only for clinical assessment and phenotypic characterisation but also for the theoretical implications concerning the relationship between sex and ASC.

As illustrated in Chapter 1, phenotypic characterisation at the behavioural and cognitive levels provides the fundamental descriptions of how males and females with ASC are similar and dissimilar to each other. This chapter focuses on comparing the core features of ASC between them. A wider range of cognitive abilities will be compared in conjunction with typical male and female adults in a two-way factorial design framework in the next chapter.

2.1.1 Sex differences in behaviour in ASC

Current international criteria for diagnosing ASC are based on behaviour (American Psychiatric Association, 2000, 2011; World Health Organization, 1992). If males and females with ASC show different behavioural phenotypes (Attwood, 2006, 2007; Gillberg, 2002; Gillberg & Coleman, 2000; Kopp & Gillberg, 1992, 2011; Skuse, 2007), we may need sex-specific behavioural criteria for defining ASC, in addition to or replacing the current criteria. This is, as was mentioned in Chapter 1, the "hard" problem of the behavioural level investigation since it requires a new definition of ASC by features other than behavioural (e.g. cognitive or neurobiological). A more conservative ("soft") approach is to start by comparing

people clinically diagnosed by the core behavioural features, an approach adopted by most studies to date including the present dissertation.

When studying sex differences in ASC there is a need for close matching in terms of age and IQ. Early studies used community or clinical samples and were not always successful in matching participants. Thus, some of the highlighted behavioural sex differences, such as greater unusual visual responses and motor stereotypy and less appropriate play in boys (Lord, et al., 1982; Volkmar, et al., 1988), and more appropriate interests (Gillberg & Coleman, 2000; Kopp & Gillberg, 1992; Wolff & McGuire, 1995) and better superficial social and communication skills in girls (Attwood, 2006, 2007; Gillberg & Coleman, 2000) may have been confounded by factors such as age or IQ.

Studies that did match the groups are inconsistent. McLennan et al. tested 21 boys and 21 girls (aged 6-36 years old) without marked intellectual disability (IQ > 60) (McLennan, et al., 1993). Boys had more severe autistic symptoms in early social communication development, measured by the Autism Diagnostic Interview (Le Couteur et al., 1989). In another example, Carter et al. found that 68 male and 22 female toddlers with ASC (aged 1.7-2.8 years old) had different cognitive and developmental profiles. Girls had better visual reception and boys had better motor and communication skills (Carter, et al., 2007). Finally, Hartley et al. tested 157 boys and 42 girls with ASC (aged 1.5-3.9 years old) using the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000) and found that girls were more impaired on the communication domain, whereas boys showed more restricted/repetitive/stereotyped interests and behaviours. Girls also had more concurrent anxious/depressed symptoms and sleep problems (Hartley & Sikora,

2009).

In contrast, other studies using matched samples report no differences between males and females with ASC. Tsai et al. found that 19 boys and 19 girls (mean age 6 years old) with classical autism were equally impaired in their cognitive, physical and self-help abilities (Tsai & Beisler, 1983). Pilowsky et al. also found no sex differences on the Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter, & Le Couteur, 1994) and the Childhood Autism Rating Scale (CARS) (Mesibov, Schopler, Schaffer, & Michal, 1989) between 18 boys and 18 girls with ASC (aged 3-30 years old) who had intellectual disability (Pilowsky, et al., 1998). Holtmann et al. matched 23 male and 23 female children and adolescents with ASC (aged 5-20.2 years old) without intellectual disability (IQ > 70, mean score 88.8) and found no differences in autistic presentation. However, females showed more parent-reported coexisting psychopathology, particularly social, attention and thought problems (Holtmann, et al., 2007). Solomon et al. matched 20 boys and 20 girls (aged 8-18 years old) with ASC and average IQ and found no differences in their ADOS scores and other autistic symptom measures including the Social Responsiveness Scale, Children's Communication Checklist 2nd Edition and the Repetitive Behaviour Scale-Revised; but in adolescence girls reported higher internalising symptoms than boys (Solomon, et al., 2011). Lastly, several questionnaire-based studies have found no evidence of behavioural sex differences in ASC (Allison, et al., 2008; Auyeung, et al., 2008; Auyeung, Wheelwright, et al., 2009; Baron-Cohen, Hoekstra, et al., 2006; Baron-Cohen, Wheelwright, Skinner, et al., 2001; J. G. Williams, et al., 2008).

These inconsistent reports on the similarities and differences between males and females with ASC may be suggestive of the marked heterogeneity of ASC, and

indicates the need to consider sub-groups stratified by age, IQ and autistic symptom severity. The demographic background of the sample population as well as the recruitment strategies may also affect the outcomes of comparisons.

2.1.2 Behavioural sex differences in adults with ASC

The above-mentioned studies all focus on children or mixed-age samples. To our knowledge there is no study addressing behavioural sex differences in *high-functioning adults with ASC*, apart from questionnaire-based studies. This is striking given the increasing awareness of the need to improve assessment, diagnosis and services for adults on the autistic spectrum (Department of Health, 2010; Murphy, et al., 2011), and given that women on the spectrum are often recognised later than males, and may be misdiagnosed (Attwood, 2006, 2007; Ernsperger & Wendel, 2007; Miller, 2003; Simone, 2010). To fill these gaps this chapter focuses on testing IQ- and age-matched male and female adults with ASC using a battery of behavioural and cognitive measures. Our intention is to extend prior questionnaire-based studies in adults to a broader range of clinical and performance-based measures of the core autistic presentation.

2.2 Methods

2.2.1 Participants

Participants were recruited through the UK Medical Research Council Autism Imaging Multicentre Study (MRC AIMS) consortium. Informed written consent was obtained for all participants in accord with procedures approved by the Suffolk

Research Ethics Committee. Between July 2007 to November 2008 (for males, recruited and tested by the Cambridge team of the MRC AIMS consortium led by Dr. Michael Lombardo) and November 2009 to October 2010 (for females, recruited and tested by myself and co-researchers), recruitment was conducted through advertisements sent to national and local autism support organisations and support groups in England and Wales, referral from diagnostic clinics for adults with autism or Asperger syndrome, and via the participant database of the Autism Research Centre, University of Cambridge (http://www.autismresearchcentre.com). The same inclusion criteria were applied to both male and female groups: aged between 18 to 45 years, with English as first language, without intellectual disability (IQ $\geq$ 70), and having a formal clinical diagnosis of autistic disorder or Asperger syndrome, based on DSM-IV (American Psychiatric Association, 2000) or ICD-10 (World Health Organization, 1992) criteria, from a chartered psychiatrist or clinical psychologist working in the UK National Health Service. Exclusion criteria for both groups included a diagnosis of current or historical psychotic disorders, substance-use disorders, medical conditions associated with autism (e.g. tuberous sclerosis, fragile X syndrome), intellectual disability, epilepsy, hyperkinetic disorder and Tourette's syndrome. Under these criteria, 83 ASC participants (45 males and 38 females) took part in a series of behavioural, cognitive, biological (genetic and salivary hormone sampling) and neuroimaging assessments at the Autism Research Centre, University of Cambridge.

2.2.2 Behavioural assessments

2.2.2.1 Subject characteristics

The main childhood caregiver of each participant was interviewed using the Autism Diagnostic Interview-Revised (ADI-R) (Lord, et al., 1994). The ADI-R is a standardised, semi-structured interview schedule based on the DSM-IV and ICD-10 diagnostic concepts of autism, exploring an individual's early development, acquisition and/or loss of language skills, language and communication functioning, social development and play, interests and behaviour, general behaviour and caregiver concerns via 93 subject items. Information used for diagnosis was based on the caregiver's report of the individual's developmental history and behaviour across time and place. On average the interview lasted 2.5 to 3.5 hours. Caregiver's descriptions of the individual's childhood (or "ever") and current behaviours were coded immediately during the interview, relying on the interviewer's judgment of the detailed descriptions of behaviours that correspond to developmental deviance. In the present study, the "diagnostic algorithm" scores were used for analysis, as most studies do, which reflect three areas of functioning: Reciprocal Social Interaction, Communication and Language, and Repetitive, Restrictive and Stereotyped Behaviour (RSB). Individuals who reached the cut-off in all the three domains, plus an onset of symptom before age of 36 months are given an ADI-R classification of "autism".

Besides the three diagnostic algorithm domain scores, for the purpose of investigating the sensory aspect, we created an "unusual sensory response" composite score from three ADI-R items that specifically addressed sensory behaviours, namely item 71 "unusual sensory interests", item 72 "undue general sensitivity to noise" and

item 73 “abnormal, idiosyncratic, negative response to specific sensory stimuli”. This composite score is the sum of the raw “ever” (i.e., lifetime) scores of the three items (raw coding of “9 = N/K or not asked” was coded as 0), giving a range of 0 to 9. Note that only item 71 contributed to the diagnostic algorithm score (for the RSB domain). Moreover, “history of language delay” was defined as either present or absent for each individual by item 9 “age of first single words” and item 10 “age of first phrases”. Individuals delayed on either or both items were defined as having a history of language delay.

All individuals with ASC were also assessed using module 4 of the Autism Diagnostic Observation Schedule (ADOS) (Lord, et al., 2000). The ADOS is a standardised activity and interview-based semi-structured assessment for current autistic behavioural presentation. Depending on the person’s expressive language level, the interviewer can administer one of the four modules. Since our participants were adults with fluent speech, module 4, consisting of 15 activities, was chosen for all participants. On average testing took 45 minutes to an hour. Behaviours of the participant during the session were recorded and coded immediately afterwards into 31 subject items, of which 16 were entered into the “diagnostic algorithm” to describe behaviour during natural interpersonal contact in the domains of Social Interaction, Communication, Imagination/Creativity and Stereotyped Behaviours and Restricted Interests. According to the coding algorithm, scores in the domains of Social Interaction, Communication, and the sum of these two contribute to the ADOS classification of “autism”, “autism spectrum” and “non-autism”. These summary scores were used for analysis, as most studies do. The ADOS has good to excellent psychometric properties, and satisfactory ability to differentiate individuals with and

without ASC (Lord, et al., 2000).

For intellectual ability, all participants were assessed by the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) that provides measures of verbal, performance and full-scale IQ.

Participants in both groups also completed three self-report questionnaires measuring their aspects of cognitive style, preferences and traits. The Autism Spectrum Quotient (AQ) (Baron-Cohen, Wheelwright, Skinner, et al., 2001) is a 50-item questionnaire measuring autistic traits in social skills, attention switching, attention to detail, communication and imagination. The Empathy Quotient (EQ) (Baron-Cohen & Wheelwright, 2004) is a 40-item questionnaire measuring thought and behavioural characteristics in both the affective and cognitive aspects of empathy. The revised Systemizing Quotient (SQ) (Wheelwright, et al., 2006) is a 75-item questionnaire measuring the cognitive and behavioural features of "systemizing", the drive to analyze, understand, predict, control and construct rule-based systems. Items in each of these questionnaires are split half between positive and negative tone to avoid response bias, and are all rated on a four-point scale from "strongly agree" to "strongly disagree".

Finally, the web version of the "Reading the Mind in the Eyes" test (Eyes Test) (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001) was completed by each participant. The Eyes Test, composed of 36 items, is an advanced mentalizing task requiring the individual to infer mental status solely from the information in photographs of a person's eyes and the immediate surrounding regions. Greyscale stimuli were presented in random order, and participant would need to choose one among four one-word response to describe the mental status of the stimuli.

The AQ, EQ, SQ and Eyes Test have excellent psychometric properties (Baron-Cohen & Wheelwright, 2004; Baron-Cohen, Wheelwright, Hill, et al., 2001; Baron-Cohen, Wheelwright, Skinner, et al., 2001; Wheelwright, et al., 2006). In addition, there are two important features of these tasks: (i) compared to typical individuals, people with ASC score significantly higher on the AQ, lower on the EQ, higher on the SQ and lower on the Eyes Test; and (ii) typical males, on average, score significantly higher on the AQ, lower on the EQ, higher on the SQ and lower on the Eyes Test compared to typical females.

2.2.2.2 Co-occurring psychiatric symptoms

Co-occurring psychiatric symptoms are not uncommon in adults with ASC (Hofvander et al., 2009), particularly depression and anxiety (Bolton, Pickles, Murphy, & Rutter, 1998; Lugnegard, Hallerback, & Gillberg, 2011; Tantam, 2000). Symptoms of anxiety and depression are also more common in females in the typical population (Crick & Zahn-Waxler, 2003). Obsessive and compulsive traits are phenomenologically related to the RSB domain of ASC and are commonly present conjointly (Russell, Mataix-Cols, Anson, & Murphy, 2005; Ruta, Mugno, D'Arrigo, Vitiello, & Mazzone, 2010). Each participant therefore filled out three well-validated, commonly used clinical and research instruments: for anxiety the 21-item Beck Anxiety Inventory (BAI) (Beck, Epstein, Brown, & Steer, 1988), for depression the 21-item Beck Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), and for obsessions and compulsive behaviours the 18-item Obsessive Compulsive Inventory-Revised (OCI-R) (Foa et al., 2002).

2.2.3 Statistical analysis

Independent samples *t*-tests were conducted to examine matching of the male and female ASC groups for age and IQ. Three separate multivariate analysis of (co)variance (MANOVA or MANCOVA) were conducted to examine childhood autistic symptoms (ADI-R algorithm domain scores), cognitive style (AQ, EQ, SQ and Eyes Test) and co-morbid psychopathology (BAI, BDI and OCI-R), respectively, in order to take into account the possible inter-dependency among the dependent variables in each cluster. Owing to the highly skewed distribution of the ADOS diagnostic algorithm scores and the ADI-R "unusual sensory response" composite score, nonparametric Mann-Whitney tests were used for these variables. Chi-square test was performed to examine the relationship between sex and history of language delay. A two-way analysis of variance (ANOVA) was then performed to examine the main effects and interaction effect of sex and history of language delay on verbal and performance IQ, respectively. All statistical analyses were performed with the PASW Statistics version 18 (SPSS Inc., Chicago, IL, USA).

2.3 Results

2.3.1 Participant characteristics

To ensure a non-biased comparison of behaviour, male and female adults are best defined as having ASC in childhood by the same behavioural criteria. To be conservative, only individuals who have available ADI-R scores and reached diagnostic algorithm cut-offs in the three domains of impaired reciprocal social interaction, communication, and repetitive, restrictive and stereotyped behaviour

(RSB) were included in the following analyses. However, failure to reach cut-off in one of the domains by one point was permitted, to allow for the possible underestimation of early developmentally atypical behaviours in the recall by caregivers whose children are now adults over the age of 18. This criterion resulted in the selection of 62 (33 males, 29 females) out of the total 83 ASC participants (45 males and 38 females) who already had a clinical diagnosis of Asperger syndrome or autistic disorder. These supra-threshold participants all scored above the cut-offs for the domains of impaired reciprocal social interaction and impaired communication, whereas 3 males (9.1%) and 6 females (20.7%) scored one point below in the RSB domain yet scored high on the other two.

The two groups were well matched on chronological age, verbal IQ, performance IQ and full-scale IQ; see Table 2-1. They were mainly young adults with average or above-average intelligence, and with similar levels of verbal and performance IQ.

Table 2-1 Age and intelligence-matched sample

	Male (N=33)	Female (N=29)	Statistics	
	Mean (SD)	Mean (SD)	*t*	*p*
Age (year)	27.0 (7.1)	26.9 (6.7)	0.085	0.933
Verbal IQ	111.5 (15.3)	113.1 (15.4)	-0.413	0.681
Performance IQ	111.1 (16.4)	109.5 (17.5)	0.373	0.711
Full-scale IQ	112.6 (16.3)	112.8 (15.7)	-0.069	0.945

SD: standard deviation

2.3.2 Childhood autistic symptoms

The first MANOVA treated sex as the only factor in the model with two levels

(i.e., male and female), and the three ADI-R diagnostic algorithm domain scores as the dependent variables. Overall, male and female adults with ASC were not significantly different from each other on childhood ADI-R scores (Wilk's lambda Λ = 0.914, $F_{(3,58)}$ = 1.826, p = 0.153). Separate univariate ANOVAs showed no significant sex differences on the reciprocal social interaction ($F_{(1,60)}$ = 0.868, p = 0.355), communication ($F_{(1,60)}$ = 2.657, p = 0.108) and RSB domains ($F_{(1,60)}$ = 4.076, p = 0.048) after Bonferroni correction for multiple comparisons; see Table 2-2. Chronological age was not correlated with any of these domain scores.

A separate Mann-Whitney test showed that females displayed significantly higher scores on "unusual sensory response" than males, with a medium effect size (female median = 3, mean = 3.1, standard deviation SD = 1.6; male median = 2, mean = 2.3, SD = 1.6; U = 321, z = 2.097, p = 0.036, Pearson r = 0.27).

2.3.3 Current interactive behaviours

Using ADOS module 4 cut-off scores to assess current symptoms, we found that 19 out of the 33 males (57.6%) and 6 out of the 29 females (20.7%) were classified as "autism spectrum" (i.e., Social Interaction + Communication scores ≥ 7); among them, 12 males (36.4%) and 4 females (13.8%) were further classified as "autism" (i.e., Social Interaction + Communication scores ≥ 10). Nonparametric Mann-Whitney tests showed that during immediate interpersonal interaction, female adults with ASC showed significantly less autistic behaviour than males in both the socio-communication (U = 251.5, z = 3.215, p = 0.001, r = 0.41) and RSB domains (U = 236.5, z = 3.931, p < 0.001, r = 0.50) with large effect sizes; see Table 2-2. Chronological age did not correlate with any of these symptom scores.

Table 2-2 Sex comparison on childhood ADI-R algorithm scores by MANOVA and current ADOS module 4 algorithm scores by Mann-Whitney tests

	Male (N=33)	Female (N=29)	Statistics		ES
	Mean (SD)	Mean (SD)	*F*	*p*	*d*
	[range]	*[range]*			
ADI-R					
Social interaction	18.0 (5.0)	16.9 (4.8)	0.868	0.355	0.22
	[10-27]	*[11-29]*			
Communication	15.2 (3.5)	13.6 (4.4)	2.657	0.108	0.41
	[8-22]	*[8-25]*			
RSB	5.7 (2.5)	4.5 (2.0)	4.076	0.048	0.53
	[2-10]	*[2-10]*			
	Median	Median	*U (z)*	*p*	*r*
	[range]	*[range]*			
ADOS module 4					
Social interaction	5 *[1-12]*	3 *[0-13]*	308 *(2.425)*	0.015	0.31
Communication	3 *[0-6]*	1 *[0-6]*	215 *(3.778)*	<0.001	0.48
S + C	7 *[1-17]*	4 *[0-19]*	251.5 *(3.215)*	0.001	0.41
RSB	1 *[0-4]*	0 *[0-1]*	236.5 *(3.931)*	<0.001	0.50

ADI-R: Autism Diagnostic Interview-Revised; RSB: repetitive, restrictive and stereotyped behaviour; ADOS: Autism Diagnostic Observation Schedule; S + C: ADOS "social interaction + communication" total scores; SD: standard deviation; ES: effect size; *d*: Cohen's *d*; *r*: Pearson *r* (small effect size, $r = 0.10 - 0.23$; medium, $r = 0.24 - 0.36$; large, $r \geq 0.37$).

2.3.4 Cognitive characteristics

A MANCOVA treated sex as the independent variable and the four measures of cognitive characteristics (AQ, EQ, SQ, Eyes Test) as the dependent variables; full-scale IQ was included as a covariate to remove variance in the data due to differences in cognitive abilities which might relate to these measures (Hoekstra,

Happé, & Ronald, 2010, conference paper presented at the BPS Developmental Psychology Section Conference, London). Overall male and female adults with ASC differed slightly in their cognitive characteristics (Wilk's lambda Λ = 0.841, $F_{(4,56)}$ = 2.648, p = 0.043). Separate univariate ANCOVAs showed that this significant difference was mainly driven by the females' reporting higher AQ, with a medium effect size ($F_{(1,59)}$ = 6.781, p = 0.012, Cohen's d = 0.65) after Bonferroni correction for multiple comparisons, whereas males and females showed comparable EQ ($F_{(1,59)}$ = 0.233, p = 0.631), SQ ($F_{(1,59)}$ = 0.856, p = 0.359) and mentalizing ability on the Eyes Test ($F_{(1,59)}$ = 0.046, p = 0.832); see Table 2-3. Chronological age was not correlated with any of these scores.

Table 2-3 Sex comparison on cognitive characteristics by MANCOVA

	Male (N=33)	Female (N=29)	Statistics		ES
	Mean (SD)	Mean (SD)	*F*	*p*	*d*
Self-reports					
AQ	32.8 (7.8)	37.6 (6.8)	6.781	0.012	0.65
EQ	20.1 (10.9)	18.9 (7.6)	0.233	0.631	0.13
SQ	66.9 (23.6)	72.5 (29.2)	0.856	0.359	0.21
Cognitive task					
Eyes Test	22.3 (5.8)	22.7 (6.6)	0.046	0.832	0.06

AQ: Autism Spectrum Quotient; EQ: Empathy Quotient; SQ: revised Systemizing Quotient; Eyes Test: correct score on the Reading the Mind in the Eyes test.

2.3.5 Co-occurring psychiatric symptoms

A significant proportion of adults with ASC showed clinically significant anxiety, depression or obsessive-compulsive symptoms; see Table 2-4. A final MANOVA

treated sex as the independent variable and the three measures of co-occurring psychiatric symptoms as the dependent variables. Overall male and female adults with ASC were not different on these symptoms (Wilk's lambda $\Lambda = 0.945$, $F_{(3,58)} = 1.127$, $p = 0.346$). Univariate ANOVAs showed no group differences on anxiety, depression or obsessive-compulsive symptoms; see Table 2-5. Chronological age was not correlated with any of these symptom scores.

Table 2-4 Severity distribution of significant co-occurring clinical symptoms

	Male (N=33) N (%)	Female (N=29) N (%)
BAI: clinically significant (score ≥ 8)	21 (63.6%)	21 (72.4%)
Mild anxiety (8-15)	8 (24.2%)	7 (24.1%)
Moderate anxiety (16-25)	11 (33.3%)	9 (31%)
Severe anxiety (26-63)	2 (6%)	5 (17.2%)
BDI: clinically significant (score ≥ 10)	18 (54.5%)	20 (69%)
Mild depression (10-18)	11 (33.3%)	10 (34.5%)
Moderate depression (19-29)	4 (12.1%)	8 (27.6%)
Severe depression (30-63)	3 (9.1%)	2 (6.9%)
OCI-R: compatible to OCD severity (score ≥ 21)	24 (72.7%)	20 (69%)

BAI: Beck Anxiety Inventory; BDI: Beck Depression Inventory; OCI-R: Obsessive-Compulsive Inventory-Revised; OCD: obsessive-compulsive disorder.

Table 2-5 Sex comparison on co-occurring clinical symptoms by MANOVA

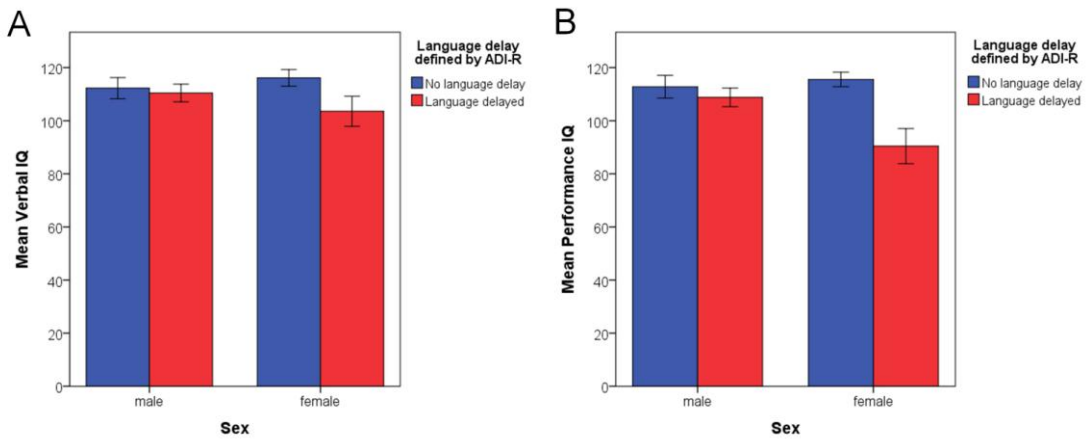
	Male (N=33) Mean (SD)	Female (N=29) Mean (SD)	Statistics *F*	*p*	ES *d*
Self-reports					
BAI	13.2 (9.9)	16.1 (10.7)	1.218	0.274	0.28
BDI	13.5 (10.4)	15.5 (8.8)	0.663	0.419	0.21
OCI-R	28.0 (12.6)	25.2 (12.3)	0.790	0.378	0.22

2.3.6 Sex difference and history of language delay

There was no association between sex and history of language delay (χ^2 = 2.304, contingency coefficient = 0.19, exact significance p = 0.18). Two-way ANOVA showed that for verbal IQ, there was no main effect of sex ($F_{(1,58)}$ = 0.124, p = 0.726) or of history of language delay ($F_{(1,58)}$ = 2.888, p = 0.095), or any interaction effect ($F_{(1,58)}$ = 1.604, p = 0.210). For performance IQ, there was no main effect of sex ($F_{(1,58)}$ = 3.289, p = 0.075), but a significant main effect of history of language delay ($F_{(1,58)}$ = 11.459, p = 0.001), and a significant interaction effect between sex and the history of language delay ($F_{(1,58)}$ = 6.024, p = 0.017). Teasing apart the interaction effect by examining each sex separately, within males with ASC we found no difference on performance IQ ($t_{(31)}$ = 0.687, p = 0.497) between those with a history of language delay (N = 14, mean = 108.8, SD = 13.0) and those without (N = 19, mean = 112.8, SD = 18.7). However, there was a large effect size for a difference in performance IQ ($t_{(27)}$ = 4.146, p < 0.001, Cohen's d = 1.80) between females with a history of language delay (N = 7, mean = 90.4, SD = 17.5) and those without (N = 22, mean = 115.6, SD = 12.8); see Figure 2-1.

Figure 2-1 Differential effects of history of language delay on IQ in male and female adults with ASC

Within adult females with ASC, those with a history of language delay showed marginally lower current verbal IQ (panel A, right bars, p = 0.053) and significantly lower current performance IQ (panel B, right bars, p < 0.001) than those without. This pattern of difference did not exist in adult males with ASC (panels A and B, left bars). Error bars represent standard error of the mean.



2.4 Discussion

This is the first study comparing behaviour in age- and IQ- matched male and female adults with high-functioning ASC. We documented important similarities and differences between the sexes. In terms of similarities, male and female adults with ASC showed comparable severity of their childhood autistic symptoms, although females self-reported more autistic traits in adulthood. We also found an absence of typical sex differences in ASC in empathizing and systemizing, and in mentalizing performance. Up to 70% of participants also fell into clinically significant ranges on

co-occurring psychopathology, a finding of importance in terms of clinical management. However, both males and females had similar levels of current co-occurring anxiety, depression and obsessive-compulsive symptoms.

In terms of differences between sexes, females presented *fewer* current socio-communication symptoms on the ADOS and had *more* lifetime sensory issues. Within females, there was also a marked difference in performance IQ in those with and without a history of language delay. This pattern of difference as a function of history of language delay was completely absent in males with ASC.

2.4.1 Similarities and differences in autistic presentation

The first important finding is the strong evidence showing *current* behavioural sex differences as measured by the ADOS. To demonstrate the importance of this marked difference, it is crucial to point out that the male and female cohorts – matched on age, verbal, performance and full-scale IQ – were not different on childhood ("most severe") core autistic symptom severity measured by the ADI-R. This implies that the two groups were "equally autistic" as children. Therefore, able adult females with ASC compared to males with ASC may achieve more progress in compensatory socio-communication ability. This may be one reason for the more marked sex difference in prevalence of ASC as the behavioural phenotype becomes milder.

One question is whether these women were true cases of ASC. Simply judging from their ADOS scores, only 6 of the 29 (20.7%) females were classified as "autism spectrum", in comparison to 19 out of the 33 (57.6%) males. However, all these females were diagnosed by experienced clinicians using DSM-IV or ICD-10 criteria,

and equally importantly, they scored above cut-off on the ADI-R. Moreover, they scored just as poorly as the males with ASC on high-level mentalizing Eyes Test (male mean score 22.3, SD 5.8; female mean score 22.7, SD 6.6). These performances are comparable to a previous independent sample of adults with ASC with similar age and IQ (mean score 21.9, SD 6.6) and are also well below the average observed in the general population (mean score 26.2, SD 3.6) and in above-average IQ controls (mean score 30.9, SD 3.0) (Baron-Cohen, Wheelwright, Hill, et al., 2001). Furthermore, these females showed the same empathizing-systemizing profile as their male counterparts. This profile is characterised as the conjunction of low empathy and high systemizing, rendering them "type S" or "extreme type S" cognitive style (Baron-Cohen, Knickmeyer, et al., 2005; Wheelwright, et al., 2006). Lastly, these females reported an even higher level of autistic traits than males and their scores were well within the range that most people with ASC typically report (Baron-Cohen, Wheelwright, Skinner, et al., 2001). All these lines of evidence support the idea that these females were not only diagnostically, neuropsychologically and cognitively on the autism spectrum (i.e., similar to the males with ASC in cognitive abilities and styles), but were also similar to their male counterparts in terms of childhood symptom severity on the ADI-R.

Whilst this study has documented that adult women with ASC present fewer current socio-communication symptoms, it is an open question as to the underlying reasons for such an effect. Our cross-sectional design is not able to address this question directly and a longitudinal study would be needed to mark developmental changes to explain such differences. However, the contrast between evident childhood symptoms and reduced current autistic interpersonal features fits with anecdotal

reports from women on the autistic spectrum (Ernsperger & Wendel, 2007; Grandin & Scariano, 1996; Miller, 2003; Simone, 2010; Willey, 1999) as well as our participants' and their caregivers' subjective experiences described in the research interviews. This suggests that able women with ASC may be more motivated and may put more effort into developing compensatory skills that help them to appear "socially typical". Hence, females with ASC may have different developmental trajectories compared to their male counterparts.

Indeed, experienced clinicians have observed that one reason females (girls or women) with ASC may be less easily identified is because of their ability to "*camouflage*" their autism (Attwood, 2006, 2007). This type of camouflaging may involve conscious, observational learning of how to act in a social setting and by adopting social roles and following social scripts (Willey, 1999). Hence, a female teenager or adult with ASC may be able to develop reciprocal conversation, social use of affect, gestures and eye gaze, that would place them under the radar for the more commonly understood and recognisable (male) phenotype of ASC (Attwood, 2007; Gillberg, 2002). Some of the women with ASC reported they could only maintain the pretence of sociability for a short conversation (15 or 20 minutes) before needing to withdraw because their 'script' did not extend to deeper or more unpredictable conversation. Some of the women consciously 'cloned' themselves on a popular girl in their class whilst at school, imitating their conversational style, intonation, movements, dress-style, interests and other mannerisms, in minute detail. This suggests that – with the right motivation – learning can be a very effective compensation strategy and could even be exploited therapeutically. Women who adopt these camouflaging strategies nevertheless report that underneath their superficially

sociable behaviour they are often experiencing high levels of stress and anxiety as they have to work hard to keep up the mask, and that it is exhausting by the end of the day.

Another suggestion is that females with ASC tend to have special interests that are less eccentric or peculiar than their male counterparts (Attwood, 2007; Gillberg, 2002; Gillberg & Coleman, 2000; Wolff & McGuire, 1995), or may simply have fewer stereotyped activities (Lord, et al., 1982; Volkmar, et al., 1988). Given the relative insensitivity of ADOS module 4 in picking up such behaviours we could not confidently confirm this possibility. However, the effect of less ADI-R RSB symptom severity in females (though not surviving correction for multiple comparisons) and our qualitative impression from interviews with caregivers was that this may be true for their behavioural presentation in childhood. From a phenotypic standpoint this is an interesting possibility and should be addressed in future research with larger samples across various ages.

Another interesting difference between females and males with ASC were the increased sensory issues in females. Although in DSM-IV sensory issues are not explicitly included in the diagnostic criteria, they are now listed as one of the key symptoms in the proposal for DSM-5 as “unusual sensory behaviours” (American Psychiatric Association, 2011). This inclusion in DSM-5 mirrors the evidence that both under- and over-responsivity to sensory stimuli may have been an overlooked feature of autism in the past (Ben-Sasson et al., 2009). Indeed, the idiosyncratic sensory and perceptual characteristics of ASC have led to hypotheses about difficulties in multisensory integration (Iarocci & McDonald, 2006), enhanced perceptual functioning (Mottron, Dawson, Soulieres, Hubert, & Burack, 2006) and the

"intense world hypothesis of autism" (Markram, Rinaldi, & Markram, 2007). More studies are needed to clarify the significance of sensory issues in ASC and its relevance to possible sex differences within ASC. One potential limitation to the observation here is that the ADI-R was not designed to be specifically sensitive to detect sensory symptoms (there are only three sensory items on the ADI-R) and only provides summary information on positive ("unusual sensory interests") and negative ("undue general sensitivity to noise" and "abnormal, idiosyncratic, negative response to specific sensory stimuli") sensory issues. Therefore, these findings should be considered preliminary.

An unexpected result that warrants further attention is the more pronounced self-reported autistic traits, as measured by the AQ, in adult females with ASC. Along with the observation of fewer current symptoms on the ADOS, these results suggest that in adulthood, females *show* fewer, but *perceive* more autistic features than males. One possible explanation for this may be that females with ASC are better at masking their autistic features, perhaps because of better self-awareness and self-referential cognitive abilities. Self-referential and social-cognitive traits are related to each other in autism (Lombardo, Barnes, Wheelwright, & Baron-Cohen, 2007), such that increases in one relates to increases in the other. Given the fewer current autistic socio-communication symptoms in females it is possible that this is indicative of some enhanced self-referential ability relative to their male counterparts. Further work testing for differences between males and females in self-referential cognition at the behavioural and neural levels (Lombardo, Chakrabarti, Bullmore, Sadek, et al., 2010) is needed. An alternative explanation could be that unlike the ADOS which is a state measure of autistic symptomatology that can be influenced by factors such as anxiety

during the interview, the AQ is a lifetime questionnaire. The AQ includes not only the state of current functioning but a generalised perception of one's own behaviour across the lifespan. It is possible that the adult females with ASC are less socially anxious during the ADOS, which may manifest in lower ADOS scores, but in fact have more autistic characteristics overall.

2.4.2 Validity of the ADOS for adults with ASC

There are several caveats in interpreting the current set of results. First, we need to consider the validity of the instruments used in this study. Although module 4 of the ADOS was originally designed to assess verbally fluent adolescents and adults, it may not be sensitive enough when used with adults in the average and above-average intelligence range who can camouflage their autistic characteristics. If an individual has learned reciprocal conversation and to use gestures, eye contact and facial expressions in social interaction adequately and frequently, s/he is unlikely to score highly on the ADOS. Yet this does not rule out the existence of other autistic features. Recent attempts to revise the ADOS diagnostic algorithm to improve validity (Gotham, Risi, Pickles, & Lord, 2007) and to create standardised ADOS scores (Gotham, Pickles, & Lord, 2009) have excluded module 4 due to the possibly distinct behavioural phenotype of adults with ASC. Furthermore, in the original psychometric study of the ADOS (Lord, et al., 2000), in module 4, only 2 out of 16 in the "autism" and 3 out of 14 in the "PDD-NOS" groups were female. In a recent validity study, although ADOS module 4 was able to discriminate ASC from psychopath and typical controls, the results were derived from male adults only (Bastiaansen et al., 2010). These suggest rather weak evidence to support the same use of the ADOS module 4 for female

adults with ASC as a tool for diagnosis. We would suggest that some tell-tale signs among females with good camouflage include speaking and/or writing too much (i.e., a pragmatics deficit) or difficulties with switching attention (e.g. talking to someone whilst composing a text message on a cell-phone). These tell-tale signs, however, warrant further testing. Researchers should use care when interpreting the results of the ADOS in assessing high-functioning adults with ASC. More research is needed to address this validity issue.

On the other hand, whilst this limitation may affect the validity of making a diagnostic judgment for ASC, it does not affect the validity of describing interactive behaviours. A sex difference in ADOS score may not be informative about their underlying diagnostic status, but is still valid in describing behaviours to certain extent. In this sense, what we observed in terms of immediate interpersonal interaction can be viewed as valid descriptions and comparisons.

2.4.3 History of language delay

The statistical interaction between history of language delay and sex on performance IQ is also noteworthy. We found that ASC female adults with a history of language delay have significantly lower performance IQ, but only marginally (non-significantly) lower verbal IQ, compared to those without this history. Interestingly this pattern was not observed in males (Figure 2-1). Although preliminary due to the small sample size of ASC females with language delay (N = 7), it raises an interesting question regarding the role of history of language delay in the development of females with ASC. On average, typical females tend to show more advanced early language development compared to males, but such a difference

normalises later in middle childhood and adolescence (Parke & Gauvain, 2008). Therefore, a delay in early language development in females with ASC may signify more severe deviance or pathology because it carries over to affect nonverbal aspects of cognition. This explanatory mechanism awaits future research.

Meanwhile, this is also informative to the ongoing debate on the revision of DSM-5 to subsume Asperger syndrome, which is characterised by normal IQ and a lack of early language delay, into the new diagnostic umbrella term of *autism spectrum disorder* (American Psychiatric Association, 2011). One major supporting argument is that by adulthood the cognitive and behavioural characteristics for people with Asperger syndrome or high-functioning autism seem largely indistinguishable (Macintosh & Dissanayake, 2004; Witwer & Lecavalier, 2008). However, all previous studies were from male-only or male-predominant samples. Our results of no correlation of early language delay with current cognitive ability in male adults fit well with the argument, yet the same might not be the case for females. Findings here suggest a crucial sex difference within ASC that early language delay may inform sub-populations of females more than of males, which should be considered in taxonomy. Furthermore, this may also explain the early reports about poorer cognitive ability in females with autism (Lord & Schopler, 1985; Lord, et al., 1982; Tsai & Beisler, 1983; Tsai, et al., 1981; Wing, 1981) – those without language delay but do have ASC may actually be frequently missed by clinicians and researchers in the past.

2.4.4 Co-occurring psychiatric symptoms

Up to 70% of these adults with ASC scored in the clinically significant range on measures of anxiety, depression and obsessive-compulsive symptoms. However, both

males and females with ASC reported comparable levels on all three measures. Obsessive-compulsive symptoms are phenomenologically related to the RSB domain of ASC and there are reports suggesting increased obsessive-compulsive symptoms in ASC compared to typical adolescents (Ruta, et al., 2010) and adults (Russell, et al., 2005). Anxiety and depression were the most prevalent co-occurring axis-1 psychiatric disorders in other independent studies of adults with ASC (Hofvander, et al., 2009; Lugnegard, et al., 2011). Clinically, close attention to these co-occurring psychiatric symptoms in both males and females with ASC is therefore essential (Tantam, 2000). Our initial look at how these might differ in males and females suggests there is no difference in the presentation of these comorbid psychopathological traits, as opposed to what was found for children and adolescents (Holtmann, et al., 2007; Solomon, et al., 2011). However, we did not include any physiological state measures related to these dimensions, which might still be different between the sexes.

2.4.5 Limitations and future directions

Because this is the first study to compare high-functioning male and female adults with ASC, it requires independent replication. Furthermore, given the substantial heterogeneity within ASC (W. Jones & Klin, 2009; Ring, Woodbury-Smith, Watson, Wheelwright, & Baron-Cohen, 2008), the focus on high-functioning adults and the conservative sample selection procedure (i.e., only those reached ADI-R cut-offs were included), one caveat is whether the results from this subgroup of adults will generalise to other subgroups such as younger individuals, those with lower IQ, those with co-occurring medical disorders or commonly associated psychiatric

conditions (e.g. fragile X syndrome, epilepsy, hyperkinetic disorder, Tourette's syndrome), or those who have mild autistic features (i.e., broader autism phenotype). Finally, participants in this study were recruited mainly from volunteer database and support groups, who are enthusiastic in helping autism research and in facilitating neuroscientists and clinicians' understanding to ASC. They are, however, not fully representative of the whole ASC community.

This chapter was set to answer the question "What are the behavioural sex differences and similarities *within* people with ASC?" Thus, it is limited in terms of the specificity in making inferences with respect to non-ASC comparison groups. The type of comparison with non-ASC groups can elucidate the main effects of sex, diagnosis, and the interaction between sex and diagnosis, which will be further reported in Chapter 3. However, the present inferences about *within*-ASC similarities and differences between the sexes still provide valuable information for a more fine-grained phenotypic comparison of male and female adults with ASC.

2.4.6 Practical implications

High-functioning male and female adults with ASC present somewhat differently in aspects of the behavioural phenotype. Although further studies are necessary to describe the *sex-general* and *sex-specific* features, practically, the implications to clinicians might be that diagnosis or phenotypic characterisation for adults assessed for possible ASC should include not only *direct interview and observation*, but also the collection of *childhood behaviour, self-report* and *neuropsychological assessment*. Judgments made only from immediate interactions might be biased due to camouflaging that may be especially pronounced in females. On the other hand,

further understanding may be gained by exploring an individual's coping mechanisms in their everyday social life. In our clinic for adults with suspected ASC, women often only reveal their difficulties in current social functioning via self-report, rather than this being immediately apparent from observation, underlining the importance of an interview with the client about her experiences and perceived difficulties, not just with an informant/parent who knew them when they were young.

Although the present design does not provide direct tests among the competing hypotheses about sex differences in terms of neurobiological and developmental mechanisms in ASC, the findings shed light on females' plausible differential presentation and developmental (compensatory) mechanisms from males, and serve as a basis for future studies. We hope the reported similarities and differences between sexes will contribute to the ongoing debates on the revision of diagnostic criteria for mental health conditions (i.e., DSM-5 and ICD-11), especially in relation to the need for better identification of females on the spectrum (Wing, et al., 2011).

Chapter 3

Do Cognitive Characteristics of ASC Vary with Sex?

3.1 Introduction

Chapter 2 has demonstrated the behavioural similarities and differences in core autistic features between male and female adults with ASC. To further understand the relationship between sex and ASC, it is crucial to investigate how males and females, with and without ASC are similar or different in other cognitive characteristics. The goal of this chapter is to disentangle this issue using a two-way factorial design analyses on six domains. ASC involves a broad range of cognitive features, from the very basic sensory perceptual processing to high-level integrative complex social cognition (Boucher, 2009). The best way to illuminate the full picture is by a wide array of cognitive tasks (Charman et al., 2011). Only six domains and limited tasks were selected here due to the limited resources of the AIMS consortium project. However they were selected based on previous classical reports on performance differences between people with and without ASC, and on the sample characteristics (i.e., adults with average or above-average IQ). The domains will be introduced successively along with brief summaries for supporting cognitive theories for autism.

3.1.1 Dispositional traits

ASC is dimensional in nature (Baron-Cohen, 2008; Wing, 1975 / 1996) and can be characterised by a broad range of behavioural and cognitive traits rather than confined symptoms. People with ASC self-perceived and reported higher *autistic traits* than the typical population (Baron-Cohen & Wheelwright, 2004; Baron-Cohen, Wheelwright, Skinner, et al., 2001; Wheelwright, et al., 2006). Parental reports for children and adolescents with ASC are consistent with this pattern (Allison, et al.,

2008; Auyeung, et al., 2008; Auyeung, Wheelwright, et al., 2009; Baron-Cohen, Hoekstra, et al., 2006; Constantino, Przybeck, Friesen, & Todd, 2000; J. G. Williams, et al., 2008). Furthermore, in the general population it has been shown that males on average have higher autistic traits than females (Allison, et al., 2008; Auyeung, et al., 2008; Baron-Cohen, Hoekstra, et al., 2006; Baron-Cohen, Wheelwright, Skinner, et al., 2001; Constantino & Todd, 2003; Posserud, et al., 2006; Ronald, Happe, Bolton, et al., 2006; Ronald, Happe, Price, et al., 2006; J. G. Williams, et al., 2008). The phenomenon that males score higher than females on measures of autistic traits, and people with ASC score even higher than typical males, has brought sex and ASC together into a special relationship (i.e., converging from two dimensions into one) which constitutes the *extreme male brain (EMB) theory of autism* at the behavioural and cognitive levels (Baron-Cohen, 2002; Baron-Cohen, Knickmeyer, et al., 2005), a formalisation of earlier similar ideas (Asperger, 1944; Wing, 1981). In this chapter we used the Autism Spectrum Quotient (AQ) (Baron-Cohen, Wheelwright, Skinner, et al., 2001), Empathy Quotient (EQ) (Baron-Cohen & Wheelwright, 2004) and the revised Systemizing Quotient (SQ) (Wheelwright, et al., 2006) to test if previous results could be replicated. We additionally used the Toronto Alexithymia Scale (TAS) (Bagby, Parker, & Taylor, 1994) to investigate traits in understanding and recognising their own emotions. Male adults with ASC show significantly higher scores than typical men on the TAS, indicating difficulties in understanding and awareness of their own emotions (Lombardo, et al., 2007).

3.1.2 Mentalizing and emotion perception

Impaired social-emotional-communication ability is the cardinal feature of ASC

(Boucher, 2009). These impairments or atypical abilities could be classified into two main aspects according to their relationship to the development of *intersubjectivity* (Trevarthen & Aitken, 2001). *Theory of mind* (ToM) or *mentalizing* deficits (of various orders) have been found throughout the life span, from the classical first-order ToM deficit in children (Baron-Cohen, Leslie, & Frith, 1985), to complex ToM deficit revealed by moral judgments (Moran et al., 2011) and deficits in spontaneous ToM (Senju, 2011) in adults with ASC. ToM/mentalizing and its antecedent *joint attention* development all involve coordinating self, other and a third party, hence they are about the aspect of *secondary intersubjectivity*. Numerous studies of ToM/mentalizing deficits as well as deviant joint attention development in children with autism (Mundy & Sigman, 1989; Mundy, Sigman, Ungerer, & Sherman, 1986) have contributed to the major theories of *mindblindness* (Baron-Cohen, 1995) and joint attention deficit (Mundy & Sigman, 1989) for ASC. In this chapter, as in Chapter 2, we used the "Reading the Mind in the Eyes" test (Eyes Test) (Baron-Cohen, Wheelwright, Hill, et al., 2001) to examine advanced mentalizing ability. It is worth noting that although in daily life females are on average better at social-emotional-communication abilities such as empathy (Baron-Cohen, 2003), and the psychometric report on the Eyes Test shows better accuracy in females than males (trend level), consistent findings confirming female advantage across mentalizing tasks are still lacking.

The other aspect of social-emotional-communication deficit in ASC is about *primary intersubjectivity*, involving abilities supporting dyadic interaction, such as face processing, emotion perception and social motivation. As early as Leo Kanner's first report he described the "autistic disturbances of *affective contact*" (Kanner, 1943), and studies in past decades have repeatedly demonstrated atypical patterns in ASC on

basic skills such as face processing (Ashwin, Wheelwright, & Baron-Cohen, 2006; Grelotti, Gauthier, & Schultz, 2002), emotion perception (Ashwin, Chapman, Colle, & Baron-Cohen, 2006) and imitation (DeMyer et al., 1972; Prior, 1979; Rogers & Pennington, 1991). These have stimulated many pivotal cognitive theories for autism, including affective contact problems and *emotion blindness* (Hobson, 1986, 1993; Kanner, 1943), primary social deficits (Fein, Pennington, Markowitz, Braverman, & Waterhouse, 1986), defective integration of affection and cognition (Ben Shalom, 2000; Hermelin & O'Connor, 1985), defective self-other mapping and self-referential cognition (Lombardo & Baron-Cohen, 2011; Rogers & Pennington, 1991), impaired interpersonal relatedness (*identification* deficit) (Hobson, 2002; Hobson & Lee, 1999; Hobson & Meyer, 2006), social orienting problem (Dawson et al., 2004), impaired (subliminal) non-verbal communication (Tantam, 2009) and problems with social motivation and reward (Grelotti, et al., 2002; Klin, Jones, Schultz, & Volkmar, 2003; Loveland, 2001; Schultz, 2005; Schultz et al., 2003). The brain basis has also been shown, such as the superior temporal sulcus for social perception (Pelphrey & Carter, 2008), the amygdala for processing negative emotions (Ashwin, Baron-Cohen, Wheelwright, O'Riordan, & Bullmore, 2007), the fusiform face area for face processing (Schultz et al., 2000) and the mirror (neuron) system for imitation (J. H. Williams et al., 2006).

Facial emotion recognition, a fundamental module serving primary intersubjectivity, is frequently reported to be atypical in ASC. Adolescents and adults with ASC demonstrate poorer performance on processing basic negative facial emotions (Ashwin, Chapman, et al., 2006; Corden, Chilvers, & Skuse, 2008; Howard et al., 2000), subtle presentations of fear (Humphreys, Minshew, Leonard, &

Behrmann, 2007), sadness (Wallace et al., 2011) and disgust (Law Smith, Montagne, Perrett, Gill, & Gallagher, 2010) as well as complex (social) emotional faces (Golan & Baron-Cohen, 2006). Nevertheless there are also reports suggesting comparable behavioural performances on basic facial emotion recognition in adolescents and adults with ASC (C. R. Jones et al., 2011; Neumann, Spezio, Piven, & Adolphs, 2006; Rutherford & Towns, 2008; Tracy, Robins, Schriber, & Solomon, 2011). The inconsistent reports on behavioural performances may be due to differences in sample demographics, task demands and outcome measures. However, studies using eye-tracking, neuroimaging and electrophysiological methods have consistently indicated atypical patterns of facial emotion processing in people with ASC (Harms, Martin, & Wallace, 2010). Here we examined the behavioural performances on facial basic emotion recognition by the Karolinska Directed Emotional Faces Test (KDEF Test), a web-based version of the original paper-and-pencil task (Ashwin, Chapman, et al., 2006). Note there has been no consensus on female superiority in the general population on facial emotion perception tasks, although report supporting this claim does exist (Campbell et al., 2002).

3.1.3 Executive function

Another cardinal feature of (though not specific to) ASC is *executive dysfunction* (Ozonoff, 1997; Ozonoff, Pennington, & Rogers, 1991; Ozonoff, Rogers, & Pennington, 1991; Ozonoff & Strayer, 1997; Rumsey, 1985; Rumsey & Hamburger, 1988). Most executive functions, including *planning*, *set-shifting*, *inhibition*, *generativity* and *self-monitoring*, have been reported to be impaired in people with ASC (Hill, 2004a). However there is inconsistency associated with experimental

designs, IQ and co-occurring conditions such as hyperkinetic disorder or Tourette's syndrome (Geurts, Corbett, & Solomon, 2009). Relatively consistent findings point to difficulties in planning, set-shifting and inhibition of prepotent response (Hill, 2004b). Working memory and simple inhibition (in a neutral condition) have generally been found typical. In this study, a simple Go/No-Go task was used to test if previous findings on simple inhibition could be replicated. On language-related executive functions, working memory was examined in the auditory domain using a phonological memory task and generativity assessed by a word generativity task. In addition, motor executive function was partially informed by a subtest of the Purdue Pegboard dexterity test (see below). Note there was no consensus indicating typical sex differences in executive functions.

3.1.4 Perceptual attention to detail

At the perceptual level, people with ASC have been reported to show preference and superior attention to detail in their behaviours (Baron-Cohen, Ashwin, Ashwin, Tavassoli, & Chakrabarti, 2009) and on visuospatial tasks (Jolliffe & Baron-Cohen, 1997; Shah & Frith, 1983). This islet superiority was initially interpreted as being a result of *weak central coherence* (WCC), i.e., defective global processing resulting in superior local processing (U. Frith, 1989). WCC theory was subsequently modified as a *preference* or *bias* towards processing parts rather than wholes in response to the emerging evidence showing intact global processing in ASC (Happe, 1999). It was then further elaborated as indicating impairments in top-down control and integrating higher-order with lower-order sensoriperceptual information (Happe & Frith, 2006). Other evidence of superior low-level processing in multiple perceptual modalities has

also contributed to the theory of *enhanced perceptual function* in autism (Mottron & Burack, 2001; Mottron, et al., 2006). Evidence from superior discrimination ability in local processing also suggested *enhanced discrimination and reduced generalisation* (Plaisted, O'Riordan, & Baron-Cohen, 1998a, 1998b). In this chapter we used the adult version of the Embedded Figures Test (EFT) (Jolliffe & Baron-Cohen, 1997; Witkin, Oltman, Raskin, & Karp, 1971) to test if previous results could be replicated. For the typical population there seems to be a male superiority in EFT performance, yet the effect size is rather small (Voyer, Voyer, & Bryden, 1995).

3.1.5 Dexterity

Although not included in the current diagnostic criteria, motor clumsiness was regarded as a diagnostic feature by Hans Asperger in his seminal report on "autistic psychopathy" (Asperger, 1944). Various motor anomalies (e.g. gross motor, fine motor, coordination, gait and posture, movement imitation) have been reported and suggested as central to ASC (D. Green et al., 2009; Jansiewicz et al., 2006; Mari, Castiello, Marks, Marraffa, & Prior, 2003; Pan, Tsai, & Chu, 2009; Provost, Lopez, & Heimerl, 2007). Rigorous meta-analysis also suggests motor coordination deficit as a pervasive, cardinal feature for ASC (Fournier, Hass, Naik, Lodha, & Cauraugh, 2010). Increasing attention is directed to its neurological and developmental implications (Jeste, 2011), as well as for early screening of ASC (Teitelbaum, Teitelbaum, Nye, Fryman, & Maurer, 1998). However there are still debates as to whether there is an intrinsic atypical motor development or that the motor clumsiness reflects executive dysfunction. In this chapter we explored dexterity and coordination using the classic Purdue Pegboard Test (Tiffin & Asher, 1948). In typical population males have been

reported to have an advantage on motor speed whereas females show an advantage on repeating a sequence after correcting for motor speed (Nicholson & Kimura, 1996).

3.1.6 Co-occurring psychiatric symptoms

Psychiatric disorders occur frequently in adults with ASC, including both high-functioning (Hofvander, et al., 2009; Lugnegard, et al., 2011) and lower-functioning populations (Tsakanikos, Underwood, Kravariti, Bouras, & McCarthy, 2011). Meanwhile sex differences in the prevalence and incidence in certain psychiatric conditions in the general population has long been noted (Bao & Swaab, 2010; Berah, 1983; Crick & Zahn-Waxler, 2003; Zahn-Waxler, et al., 2008), particularly the more frequent later-onset affective disorders in females and early-onset neurodevelopmental conditions in males. How this sex bias is modified by ASC is, however, rarely investigated. In adolescence it has been shown that teenage girls with ASC report higher internalising symptoms than both teenage boys with ASC and typical teenage girls; meanwhile, teenage boys with ASC do not differ significantly from typical teenage boys apart from marginally higher depressive symptoms (Solomon, et al., 2011). In adults the rates of comorbid diagnoses seem to not differ between males and females with ASC (Hofvander, et al., 2009; Lugnegard, et al., 2011), though females more often report being bullied at school (Hofvander, et al., 2009). As in Chapter 2, we used the Beck Anxiety Inventory (BAI) (Beck, et al., 1988), Beck Depression Inventory (BDI) (Beck, et al., 1961) and Obsessive Compulsive Inventory-Revised (OCI-R) (Foa, et al., 2002) to assess these commonly co-occurring psychiatric symptoms.

3.2 Methods

3.2.1 Participants

Participants were recruited through the UK Medical Research Council Autism Imaging Multicentre Study (MRC AIMS) consortium, details given in Chapter 2. Alongside the ASC groups (45 males and 38 females), 33 typical male (recruited and tested by the Cambridge team of the MRC AIMS consortium led by Dr. Michael Lombardo) and 35 typical female adults (recruited and tested by myself and co-researchers) also took part in the study, who met the same exclusion criteria as for the ASC groups, in addition to not having a diagnosis of ASC themselves and in their family history.

3.2.2 Measures

3.2.2.1 Dispositional traits

Details of AQ, EQ and SQ were already given in Chapter 2. The Toronto Alexithymia Scale (TAS) is a 20-item self-report questionnaire on one's self-awareness of own emotions.

3.2.2.2 Mentalizing and emotion perception

For advanced mentalizing ability, details of the Eyes Test were given in Chapter 2. Both the correct score and reaction time (RT) were taken as outcome variables. However, due to the high cognitive demands of the task, the correct score was considered more informative. RT was positively skewed. For parametric statistics to be applicable it was log-transformed to approximate normal distribution.

The web version of the Karolinska Directed Emotional Faces Test (KDEF Test) was implemented to measure basic emotion perception. The KDEF Test is a 140-item basic emotion recognition task based on the KDEF photograph set of static faces (Lundqvist, Flykt, & Ohman, 1998), comprised of seven sets of 20 colour faces presenting six basic emotions (i.e., happy, sad, angry, fear, disgusted, surprised) and a neutral expression, with all stimuli presented in random order. Participants were asked to choose one among seven responses to identify the emotion of the face stimuli by pressing number 1-7 on the keyboard, and were instructed to be as fast and as accurate as possible. Due to potential ceiling effect in accuracy, we opted for reaction time but calculated *accuracy-adjusted reaction time* (aaRT = mean reaction time / accuracy) for each emotion to account for performance information on both (Sutherland & Crewther, 2010). The seven aaRTs were all positively skewed so were log-transformed to approximate normal distribution. There were still few right-tail outliers for these log-transformed aaRTs[2], so *winsorising* was further performed as a trial by moving all outliers to two standard deviations above the mean. This procedure, however, did not change any of the outcomes of statistical comparisons so results from the non-winsorised aaRTs will be reported[3].

---

[2] Outliers (all/extreme) were identified by each group. There were in total 0/0 for happy, 2/0 for sad, 4/3 for angry, 5/1 for fear, 5/1 for disgusted, 6/2 for surprised and 3/0 for neutral faces. There was no individual being an outlier in all or most of the emotions. This indicated that the outlier was poor in recognising particular emotion(s) but not generally slow/inaccurate.

[3] There are other reasons for this decision besides the same group comparison results on winsorised versus non-winsorised data. As pointed out above, the outlier aaRT contains information of the emotion recognition ability on a specific emotion but not generally all emotions, reflecting the participant's actual ability on a particular emotion. Winsorising may reduce the extent of violation to assumptions for parametric tests, but as a consequence the information about particularly poor performance on a

3.2.2.3 Executive function

A web version simple Go/No-Go task was used to assess simple inhibition in neutral condition. Participants were instructed to press the "left key" (Q at the left side of the keyboard) by the left hand when seeing a bold arrow pointing to the left presented on the screen, the "right key" (P at the right side of the keyboard) by the right hand when seeing an arrow pointing to the right, and to not respond when seeing an arrow pointing upward. A total of 300 stimuli (110 left, 110 right, and 80 upward) were presented randomly. Reaction time and response for all 300 items were recorded. Results were first explored by calculating the classic commission error (press "left" or "right" when the stimuli is upward and should be ignored) and omission error (no response when "left" or "right" should be pressed). Due to the highly skewed distribution, these two error rates were first rank-transformed to approximate normal distribution for analysis. The results were then re-analysed under the framework of signal detection theory (SDT) (D. M. Green & Swets, 1966) to estimate two major parameters: *sensitivity* ($d' = Z_{Hit} - Z_{FA}$, where Z_{Hit} is the corresponding Z value in the normal distribution for the probability of *Hit* [i.e., signal present and the response is "present"], and Z_{FA} the same for *False Alarm* [i.e. signal absent but the response is "present"]) and *criterion* ($C = -0.5 \times (Z_{Hit} + Z_{FA})$). *Sensitivity d'* indicates the participant's ability to discriminate signal from noise, and *criterion C* quantifies how liberal (i.e., $C < 0$) or conservative (i.e., $C > 0$) the response strategy / bias is. *d'* and *C* were normally distributed so no further transform was performed.

---

specific emotion, which is very informative for group comparisons, may get lost. We decided to report the results from the non-winsorised data also because F-test is robust to violations to assumptions (Glass, Peckham, & Sanders, 1972).

Two language-related executive functions were assessed. Phonological memory was with the Non-Word Repetition task (Gathercole, Willis, Baddeley, & Emslie, 1994), consisting of 28 non-words. Participants were asked to closely listen to a non-word (in a British English accent) and to repeat it immediately. Their utterance was recorded and coded by a trained native British researcher using strict criteria that all vowels, consonants and accents of the repetition need to be exactly the same as the stimulus for the item to be coded as correct. Number of correct items was treated as the outcome variable. The word generativity task required the participant to produce as many words beginning with the letter "F" as possible in one minute. Names, tense changes, plurals, derivatives and pronouns were not allowed. The same task was then performed with letters "A" and "S". Total words generated, excluding repetitions and those breaking rules, were treated as the outcome variable.

Motor executive function involving motor coordination, inhibition and planning was partially assessed by the 'assembly' subtask of the Purdue Pegboard Test (Tiffin & Asher, 1948); see below (section 3.2.2.5) for detail.

3.2.2.4 Perceptual attention to detail

In this study we used the adult version of the Embedded Figures Test (EFT) (Witkin, et al., 1971). Similar to a previous study (Jolliffe & Baron-Cohen, 1997), we used Form A which consisted of 12 figures in fixed order plus an additional practice item, each depicting a complex design and a simple shape which was hidden in the complex design. The participant was first shown and asked to study the complex design for no longer than 15 seconds, then shown the simple shape (meanwhile the complex design covered) for no longer than 10 seconds. Timing (using a stopwatch) started when the complex design was shown again to the participant (meanwhile the

simple shape covered) and s/he would need to identify the simple shape with a stylus pen. Time was noted (but not stopped) once the participant said s/he found the simple shape. If the answer was correct, the time was recorded. If the answer was incorrect then s/he was asked to find it again, and the final time was recorded when the tracing was correct. Participants were given an upper limit of 120 seconds, and failure to find the simple shape within this allotted time was scored as a failure and the response time for the item was recorded as 120 seconds.

Two analysis strategies were employed, accounting for different aspects of the task performance (White & Saldana, 2011). First, average response time from all 12 items (including both correct and failure items) were used as the outcome measure, in order to account for both accuracy and response time[4]. Second, to purely assess performance speed on correct items, mean response time for correct items only were taken as the outcome measure; in the following statistical modelling, accuracy was included as a covariate to reduce the influence of accuracy on response time (as suggested by White & Saldana, 2011).

3.2.2.5 Dexterity

The Purdue Pegboard Test (Tiffin & Asher, 1948) is a highly reliable and well-validated standard test for dexterity, involving both gross movement of arms, hands and fingers and fingertip dexterity. The test consists of four parts: (i) right hand: the participant was asked to insert small pins into holes on the board for 30 seconds using only their right hand, and the number of pins successfully placed in the holes

[4] This strategy was often used in previous studies (Jarrold, Gilchrist, & Bender, 2005; Jolliffe & Baron-Cohen, 1997; Kaland, Mortensen, & Smith, 2007; Ropar & Mitchell, 2001).

were scored; (ii) left hand: the same procedure was repeated using the left hand; (iii) both hands: the same procedure was used but the participant was instructed to pick up and place pins in two rows of holes using both hands alternatively; and (iv) assembly: using both hands alternatively, the participant was asked to assemble sequences of pins, collars and washers for 60 seconds, and the number of all items successfully placed in the holes were recorded.

3.2.2.6 Co-occurring psychiatric symptoms

Details of the BAI, BDI and OCI-R were given in Chapter 2.

3.2.3 Statistical analysis

In order to investigate the relationship between sex and ASC, all analyses were performed under a two-way factorial analysis of variance framework, with sex and ASC as the two fixed factors, each with two levels. Due to potential interdependency amongst the outcome variables within each domain or outcome measures from the same task, multivariate analysis of (co)variance (MAN(C)OVA) was first conducted for each domain or outcome measures from the same task, followed by individual AN(C)OVA if the overall MAN(C)OVA yielded significant results. The alpha level for these post-hoc AN(C)OVAs were corrected for multiple comparisons using the Bonferroni method within each MAN(C)OVA (i.e., 0.05 / number of AN(C)OVAs performed). For each AN(C)OVA, if significant, four planned pair-wise[5] comparisons

[5] The term *pair-wise* used throughout this dissertation indicates a planned comparison between two independent groups, but *not* "paired (dependent) groups". The statistical tests performed all treated the two groups as independent.

were performed between (i) males with and without ASC, (ii) females with and without ASC, (iii) typical control males and females, and (iv) males and females with ASC; alpha level was set at 0.05 / 4 = 0.0125 by Bonferroni correction.

Age was found to be correlated with most of the reaction time-derived measures (the older the slower), and IQ with most accuracy measures (the higher the more accurate). Therefore for the cognitive tasks (i.e., mentalizing and emotion perception, executive function, perceptual attention to detail and dexterity) analyses were performed with age and FIQ as covariates. As a supplement, we also performed all the tests without covarying age and FIQ, and found the group difference patterns and their significance stayed the same before and after including the covariates. Results from models covarying with age and FIQ are reported. All statistical analyses were performed with the PASW Statistics version 18 (SPSS Inc., Chicago, IL, USA).

3.3 Results

3.3.1 Participant characteristics

The ASC participants were selected according to the procedures described in Chapter 2, conservatively based on their ADI-R scores. Thirty-three male and 29 female adults scored above the threshold. Another three women, though not having ADI-R data available (because their childhood caregivers were not able to provide the interview), were also included for the reasons that one scored above the cut-off for 'autism spectrum' on the ADOS, one previously received a diagnosis using the Adult Asperger Assessment (AAA) (Baron-Cohen, Wheelwright, Robinson, & Woodbury-Smith, 2005) which had incorporated care-giver reports on childhood

behaviours, and one's diagnosis received from an expert clinician (Professor Digby Tantam, University of Sheffield) with assessments including comprehensive childhood developmental history. This resulted in 32 females with ASC included in the analysis. In order to match for age, IQ and group size, the final cohort consisted of 32 participants per group.

Table 3-1 shows the means, standard deviations and group comparison results. All groups (MC: typical control male adults; MA: male adults with ASC; FC: typical control female adults; FA: female adults with ASC) were pair-wise matched on age and full-scale IQ. For subscales, FC scored higher than MC on verbal IQ and MC scored higher than FA on performance IQ under a non-corrected threshold of $p < 0.05$. Similar to the pattern observed in Chapter 2, for ADI-R MA scored slightly higher than FA in RSB domain but they had the same severity on social interaction and communication domains. MA scored significantly higher than FA in ADOS scores even after multiple comparison correction.

Table 3-1 Demographic and behavioural characteristics for the four groups

	MC (N=32) Mean (SD)	MA (N=32) Mean (SD) [range] $	FC (N=32) Mean (SD)	FA (N=32) Mean (SD) [range] $	Statistics &
Age (Years)	28.7 (5.9)	27.0 (7.2)	27.6 (6.3)	28.1 (8.2)	ns
Verbal IQ	111.0 (12.2)	112.5 (14.4)	118.3 (10.1)	114.5 (15.4)	FC>MC (p = .030)
Performance IQ *	118.3 (11.5)	112.2 (15.3)	116.4 (9.4)	110.2 (17.0)	MC>FA (p = .019)
Full IQ *	116.3 (11.8)	113.7 (15.1)	119.7 (8.4)	114.1 (15.5)	ns
ADI-R #					
Social	–	18.0 (5.1) [10 – 27]	–	16.9 (4.8) [11 – 29]	ns
Communication	–	15.2 (3.5) [8 – 22]	–	13.6 (4.4) [8 – 25]	ns
RSB	–	5.8 (2.5) [2 – 10]	–	4.5 (2.0) [2 – 10]	MA>FA (p = .035)
ADOS @					
S + C	–	8.5 (4.8) [1 – 17]	–	4.6 (4.4) [0 – 19]	MA>FA (p < .001)
RSB	–	1.0 (1.0) [0 – 4]	–	0.1 (0.3) [0 – 1]	MA>FA (p < .001)

$: For ADI-R and ADOS scores.

&: Independent sample *t*-tests. All *p* values were **not** corrected for multiple comparisons.

*: Levene's Test for Equality of Variances showed significant non-equal variances, therefore equal variance was not assumed in the statistical tests.

#: N = 32 for MA, N = 29 for FA.

@: Distribution of scores significantly deviant from normal, therefore non-parametric Mann-Whitney tests were performed for group comparison of ADOS algorithm scores.

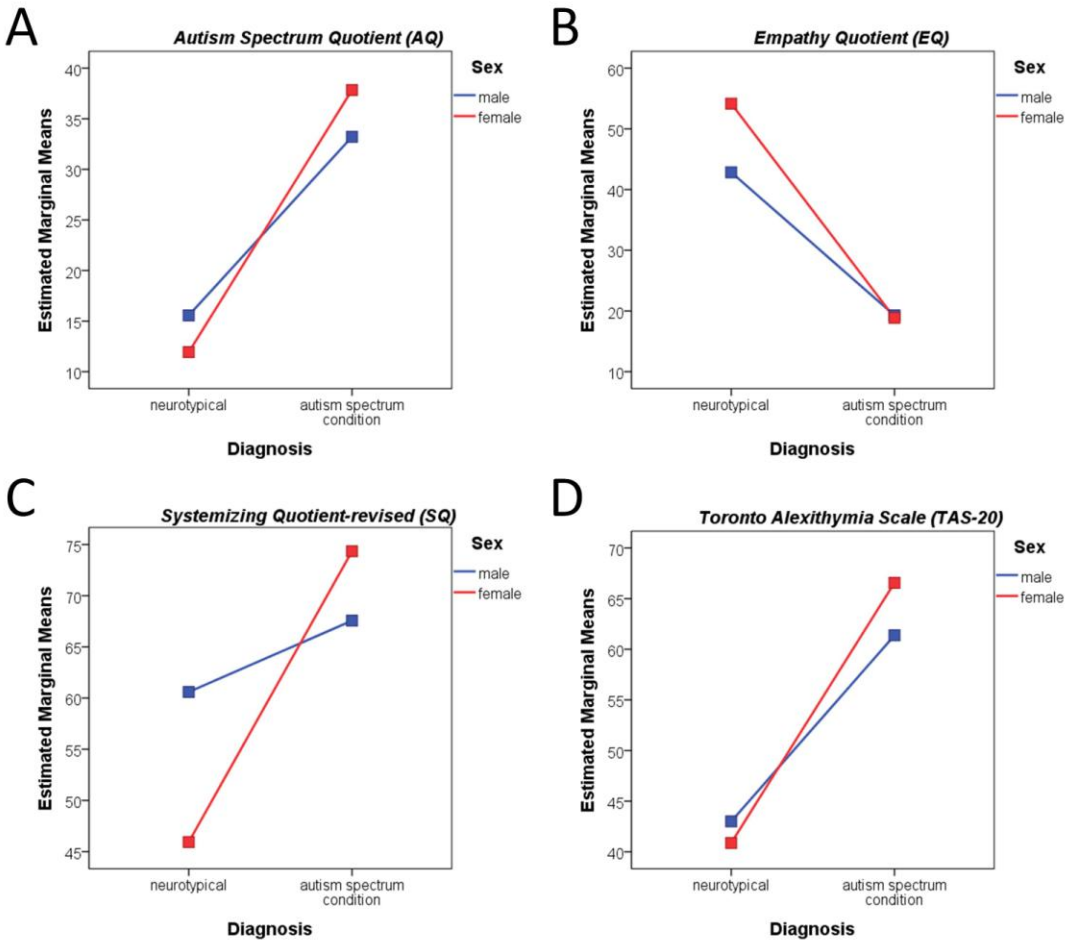
MC = (neuro)typical control group male adults; MA = male adults with ASC; FC = (neuro)typical control group female adults; FA = female adults with ASC; SD = standard deviation; ns = non-significant ($p > 0.05$); ADI-R = Autism Diagnostic Interview-Revised; RSB: repetitive, restrictive and stereotyped behaviour; ADOS: Autism Diagnostic Observation Schedule; S + C: ADOS "social interaction + communication" total scores.

3.3.2 Dispositional traits

The first MANOVA treated the four dispositional traits (AQ, EQ, SQ and TAS scores) as dependent variables, and sex (two levels: male and female) and diagnosis (two levels: neurotypical control and ASC) as fixed factors. There was a significant main effect of sex (Pillai's Trace $V = 0.140$, $F_{(4,121)} = 4.922$, $p = 0.001$, $\eta_p^2 = 0.140$), a significant main effect of diagnosis (Pillai's Trace $V = 0.792$, $F_{(4,121)} = 115.445$, $p < 0.001$, $\eta_p^2 = 0.792$) and a significant sex-by-diagnosis interaction effect (Pillai's Trace $V = 0.121$, $F_{(4,121)} = 4.161$, $p = 0.003$, $\eta_p^2 = 0.121$). Post-hoc univariate two-way factorial ANOVAs showed that after Bonferroni correction for multiple comparisons, the main effect of sex was evident only for EQ ($F_{(1,124)} = 9.841$, $p = 0.002$, $\eta_p^2 = 0.074$); the main effect of diagnosis was evident universally for all four measures, including AQ ($F_{(1,124)} = 361.365$, $p < 0.001$, $\eta_p^2 = 0.745$), EQ ($F_{(1,124)} = 286.470$, $p < 0.001$, $\eta_p^2 = 0.698$), SQ ($F_{(1,124)} = 17.937$, $p < 0.001$, $\eta_p^2 = 0.126$) and TAS ($F_{(1,124)} = 164.465$, $p < 0.001$, $\eta_p^2 = 0.570$). The interaction effect was evident for AQ ($F_{(1,124)} = 12.961$, $p < 0.001$, $\eta_p^2 = 0.095$), EQ ($F_{(1,124)} = 11.362$, $p = 0.001$, $\eta_p^2 = 0.084$) and SQ ($F_{(1,124)} = 6.587$, $p = 0.011$, $\eta_p^2 = 0.050$). The line graphs (Figure 3-1) revealed that the significant interactions were *ordinal*, in which case the significant main effects of diagnosis could be interpreted.

Figure 3-1 Dispositional traits of the four groups

Line graphs (panels A, B, C, D illustrate AQ, EQ, SQ, TAS, respectively) indicate the interrelationship amongst the four groups under a two-way factorial ANOVA framework. The squares indicate the estimated marginal mean for each group on the y-axis. The x-axis illustrates diagnostic group with the neurotypical control group on the left and ASC group on the right. The colours indicate sex with blue representing males and red representing females. EMB pattern was evident for AQ, EQ and SQ because there was a typical sex difference, alongside a main effect of ASC diagnosis that conformed to the pattern predicted by the EMB model (i.e., ASC – male – female).



The significant main effects indicated that altogether males and females with ASC scored lower on EQ and higher on AQ, SQ and TAS than typical controls. To interpret the sex-by-diagnosis interactions for AQ, EQ and SQ, planned pair-wise comparisons (after Bonferroni correction) revealed that in the control groups, males scored higher on AQ (marginal, $p = 0.015$), SQ ($p = 0.004$) and lower on EQ ($p < 0.001$) than females. However, in the ASC group, although they all scored significantly differently from the controls, the typical sex differences disappeared for EQ ($p = 0.855$) and SQ ($p = 0.320$), and even reversed for AQ where women with ASC scored higher than men with ASC ($p = 0.011$). These observations generally fit the predictions of the EMB theory.

3.3.3 Mentalizing and emotion perception

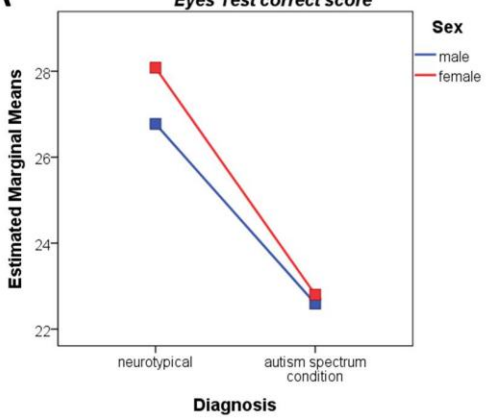
A MANCOVA treated nine outcome measures from the Eyes Test and KDEF Test (i.e., Eyes Test correct score, log-transformed Eyes Test RT, and log-transformed aaRT for the seven emotions in KDEF Test) as dependent variables, sex and diagnosis as fixed factors, and age and FIQ as covariates. There was a significant main effect of diagnosis (Pillai's Trace $V = 0.380$, $F_{(9,114)} = 7.773$, $p < 0.001$, $\eta_p^2 = 0.380$) but not of sex, nor significant interaction. Post-hoc univariate two-way factorial ANCOVAs showed that the main effect of diagnosis was evident for all measures except the log-transformed Eyes Test RT: Eyes Test correct score ($F_{(1,122)} = 30.295$, $p < 0.001$, $\eta_p^2 = 0.199$), log-transformed aaRT for happy ($F_{(1,122)} = 38.947$, $p < 0.001$, $\eta_p^2 = 0.242$), sad ($F_{(1,122)} = 24.511$, $p < 0.001$, $\eta_p^2 = 0.167$), angry ($F_{(1,122)} = 11.431$, $p = 0.001$, $\eta_p^2 = 0.086$), fear ($F_{(1,122)} = 24.413$, $p < 0.001$, $\eta_p^2 = 0.167$), disgusted ($F_{(1,122)} = 15.796$, $p < 0.001$, $\eta_p^2 = 0.115$), surprised ($F_{(1,122)} = 17.189$, $p < 0.001$, $\eta_p^2 = 0.123$) and neutral

($F_{(1,122)}$ = 14.269, p < 0.001, η_p^2 = 0.105) faces. It was also confirmed that there was no significant main effect of sex or sex-by-diagnosis interaction on any ANCOVA. This indicated in general, people with ASC were less accurate in advanced mentalizing and slower in identifying all seven basic facial emotions; see Figure 3-2. Post-hoc planned pair-wise comparisons confirmed the presence of significant simple effects of diagnosis across both sexes for all variables, except non-significance in females for disgusted, surprised and neutral faces (panels F, G, H). Furthermore, there were no sex differences in the typical groups.

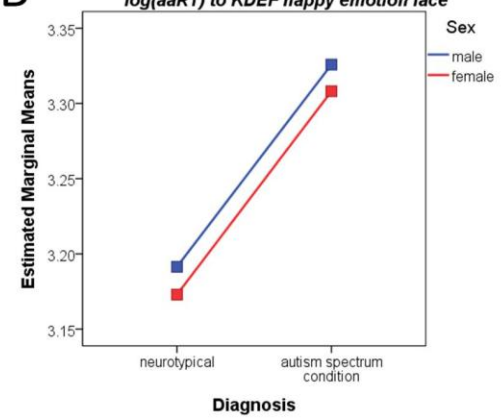
Figure 3-2 Eyes Test and KDEF Test performances of the four groups

The line graphs illustrate the main effects of diagnosis across outcome variables (A: Eyes Test correct score; B-H: log-transformed aaRT for KDEF Test emotion faces of happy, sad, angry, fear, disgusted, surprised and neutral faces). There were no sex-by-diagnosis interactions. Convention of the graphs is the same as that in Figure 3-1.

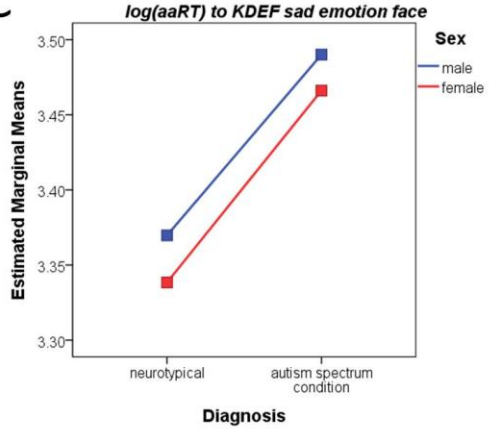
A


Eyes Test correct score
Sex
male
female
Estimated Marginal Means
28
26
24
22
neurotypical
autism spectrum condition
Diagnosis

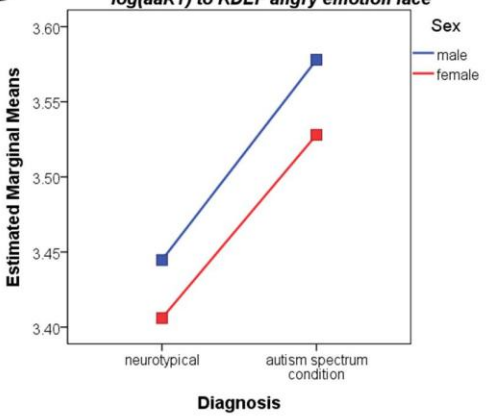
B


log(aaRT) to KDEF happy emotion face
Sex
male
female
Estimated Marginal Means
3.35
3.30
3.25
3.20
3.15
neurotypical
autism spectrum condition
Diagnosis

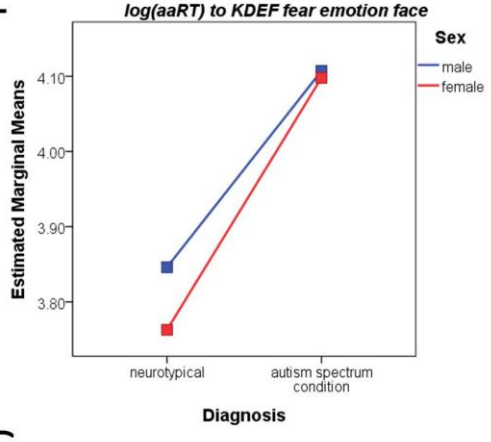
C


log(aaRT) to KDEF sad emotion face
Sex
male
female
Estimated Marginal Means
3.50
3.45
3.40
3.35
3.30
neurotypical
autism spectrum condition
Diagnosis

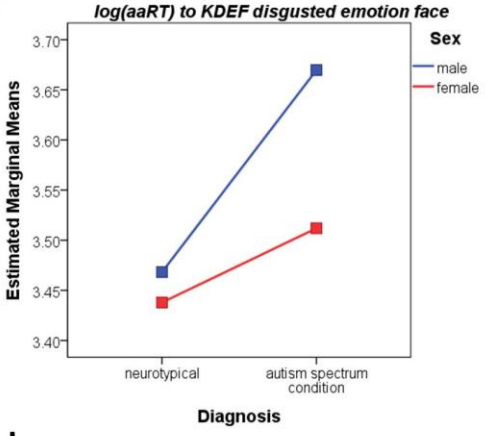
D


log(aaRT) to KDEF angry emotion face
Sex
male
female
Estimated Marginal Means
3.60
3.55
3.50
3.45
3.40
neurotypical
autism spectrum condition
Diagnosis

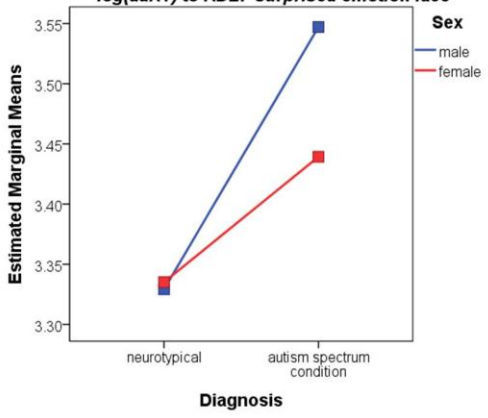
E


log(aaRT) to KDEF fear emotion face
Sex
male
female
Estimated Marginal Means
4.10
4.00
3.90
3.80
neurotypical
autism spectrum condition
Diagnosis

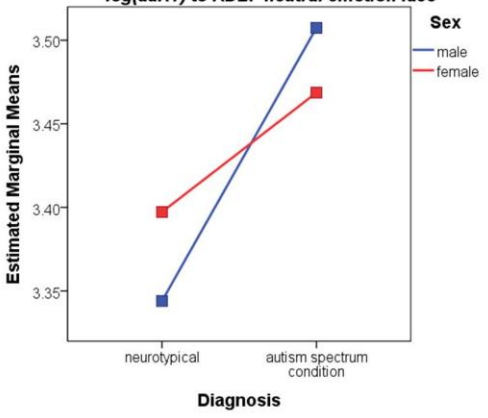
F


log(aaRT) to KDEF disgusted emotion face
Sex
male
female
Estimated Marginal Means
3.70
3.65
3.60
3.55
3.50
3.45
3.40
neurotypical
autism spectrum condition
Diagnosis

G


log(aaRT) to KDEF surprised emotion face
Sex
male
female
Estimated Marginal Means
3.55
3.50
3.45
3.40
3.35
3.30
neurotypical
autism spectrum condition
Diagnosis

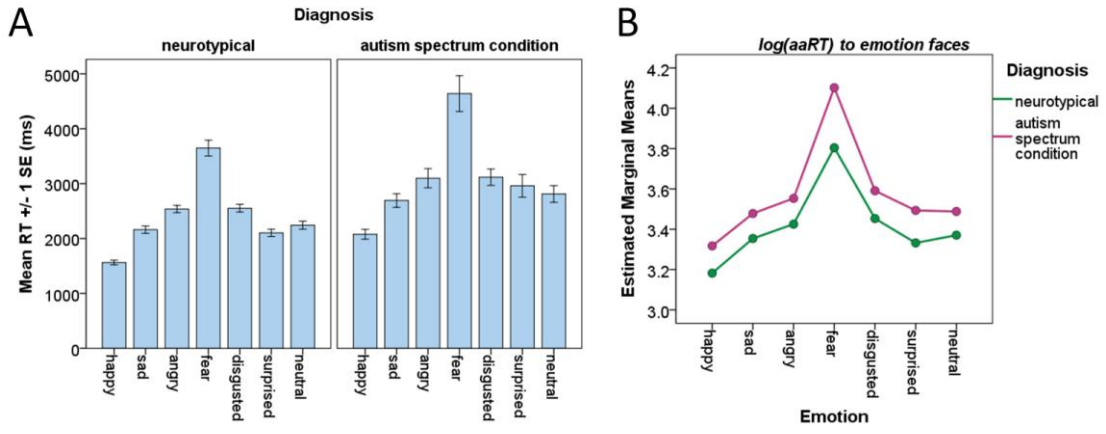
H


log(aaRT) to KDEF neutral emotion face
Sex
male
female
Estimated Marginal Means
3.50
3.45
3.40
3.35
neurotypical
autism spectrum condition
Diagnosis

To further explore if any particular emotion is specifically difficult for people with ASC to identify, a mixed ANCOVA was performed treating emotion as the within-subject factor (with seven levels), sex and diagnosis as the between-subject (fixed) factors, and age and FIQ as covariates. Results showed a significant emotion-by-diagnosis interaction ($F_{(3.149, 384.133)} = 3.854$, $p = 0.009$; *df* was corrected using Greenhouse-Geisser estimates of sphericity due to significant violation of the assumption of sphericity, Mauchly's test $p < 0.001$), but no emotion-by-sex or emotion-by-sex-by-diagnosis interactions. Contrasts revealed that people with ASC were particularly slower than controls in identifying fear compared to either neutral ($F_{(1,122)} = 6.826$, $p = 0.01$) or happy ($F_{(1,122)} = 7.341$, $p = 0.008$) faces; see Figure 3-3.

Figure 3-3 KDEF Test emotion-by-diagnosis interaction

Panel A depicts the raw reaction times across all seven emotion faces for the neurotypical control group (left graph) and the ASC group (right graph). For both groups, fear (4th bars) required the longest time to identify, and happy (1st bars) the shortest. Error bars indicate standard error of the mean. Panel B indicates that the ASC groups (purple line) were different from the control groups (green) in that they required an even longer time to identify fear compared to neutral or happy faces. There was no sex difference in this emotion-by-diagnosis interaction so males and females are illustrated altogether.



3.3.4 Executive function

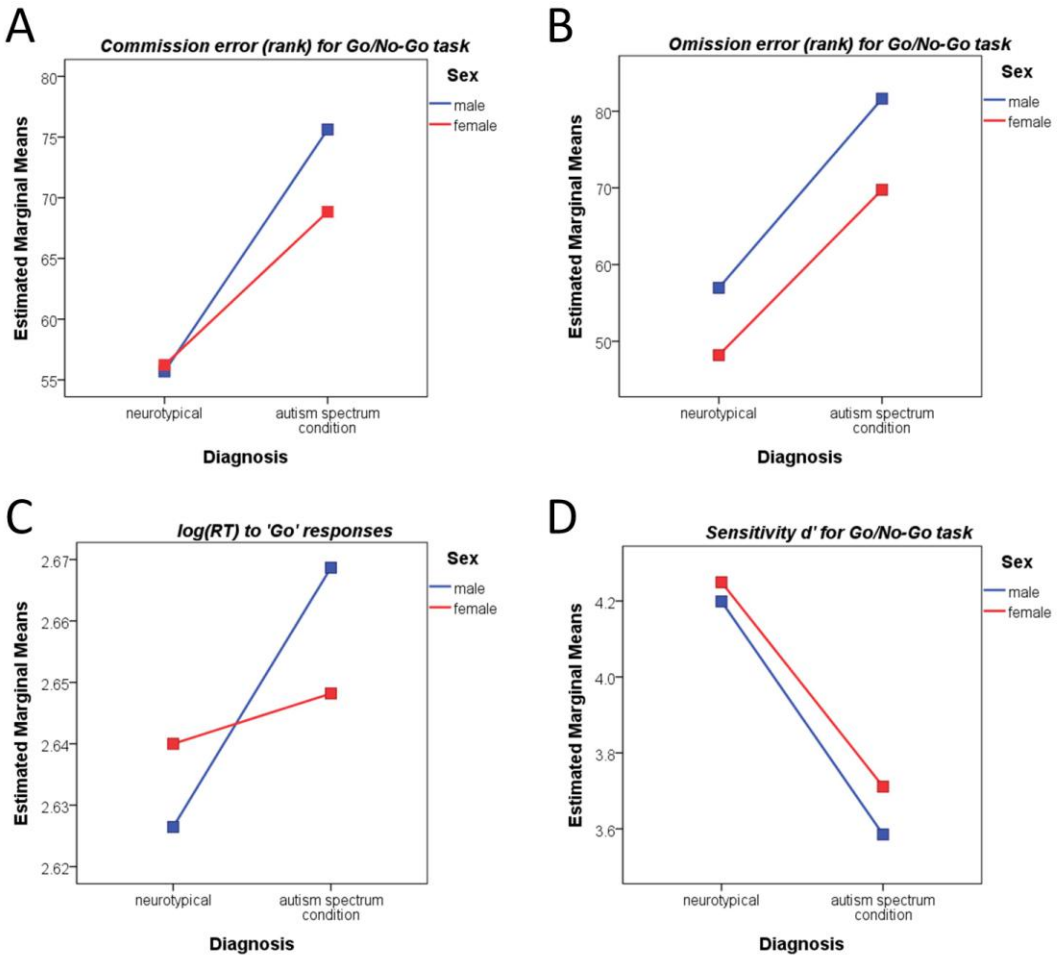
The Go/No-Go data for one male ASC participant was not recorded due to website failure, so there were only 31 male participants included in this analysis. We first performed an exploratory analysis on the conventional outcome measures of commission and omission errors. A MANCOVA treated the two rank-transformed error rates as dependent variables, sex and diagnosis as fixed factors, and age and FIQ as covariates. There was a significant main effect of diagnosis (Pillai's Trace V = 0.137, $F_{(2,120)}$ = 9.490, p < 0.001, η_p^2 = 0.137) but not of sex, nor a significant interaction. Post-hoc univariate two-way factorial ANCOVAs showed that the main effect of diagnosis (i.e., people with ASC had more errors) was evident for both measures: rank-transformed commission error ($F_{(1,121)}$ = 6.510, p = 0.012, η_p^2 = 0.051) and rank-transformed omission error ($F_{(1,121)}$ = 17.254, p < 0.001, η_p^2 = 0.125). It was also confirmed that there was no significant main effect of sex or sex-by-diagnosis interaction on either ANCOVA. Post-hoc planned comparisons showed that the simple effect of diagnosis was significant across sexes for omission error (in males p = 0.001, in females p = 0.010), but not for commission error in either sex (in males p = 0.022, in females p = 0.235). See Figure 3-4, panels A and B.

Data were re-analysed with measures derived from SDT. A MANCOVA treated the log-transformed mean RT for all "go" trials, sensitivity *d'* and criterion *C* as dependent variables, sex and diagnosis as fixed factors, and age and FIQ as covariates. Echoing the exploratory analysis, there was a significant main effect of diagnosis (Pillai's Trace V = 0.162, $F_{(3,119)}$ = 7.688, p < 0.001, η_p^2 = 0.162) but not of sex, nor was there a significant interaction. Post-hoc ANCOVAs showed that the main effect of diagnosis was only evident for log-transformed mean "go" RT ($F_{(1,121)}$ = 7.329, p =

0.008, η_p^2 = 0.057) and *d'* ($F_{(1,121)}$ = 12.748, p = 0.001, η_p^2 = 0.095), but not for *C*. There was no significant main effect of sex or sex-by-diagnosis interaction on any ANCOVA. This indicated in general, people with ASC were slower in response to stimuli and less sensitive in discriminating signal from noise, yet their response style/bias was similar to controls. Post-hoc comparisons showed that the simple effect of diagnosis was significant in males but not in females for "go" RT (in males p = 0.001, in females p = 0.458) and *d'* (in males p = 0.001, in females p = 0.053); note that these differences did not reach a significant sex-by-diagnosis interaction. See Figure 3-4, panels C and D.

Figure 3-4 Go/No-Go task performance of the four groups

The line graphs illustrate main effect of diagnosis across outcome variables (A, B: rank-transformed commission and omission errors, the higher the more error; C: log-transformed RT for "go" response; D: sensitivity d' derived from SDT). There were no sex-by-diagnosis interactions. Convention of the graphs is the same as that in Figure 3-1.



Another MANCOVA was conducted for the language-related executive functions, treating Non-Word Repetition (NWR) and word generativity (FAS) task scores as the dependent variables, sex and diagnosis the fixed factors, and age and FIQ the

covariates. There was a significant main effect of sex (Pillai's Trace $V = 0.067$, $F_{(2,121)} = 4.337$, $p = 0.015$, $\eta_p^2 = 0.067$) but not of diagnosis, nor was there a significant interaction. Post-hoc ANCOVAs showed that the main effect of sex was only evident for FAS ($F_{(1,122)} = 6.509$, $p = 0.012$, $\eta_p^2 = 0.051$) but not for NWR scores, and confirmed no main effect of diagnosis or interaction effect in either of them.

3.3.5 Perceptual attention to detail

An ANCOVA was performed for the *mean RT for all items* in EFT, with sex and diagnosis as fixed factors and age and FIQ as covariates. We noted significant main effects of sex ($F_{(1,122)} = 22.944$, $p < 0.001$, $\eta_p^2 = 0.158$) and diagnosis ($F_{(1,122)} = 5.538$, $p = 0.020$, $\eta_p^2 = 0.043$) and a marginal interaction effect ($F_{(1,122)} = 3.700$, $p = 0.057$, $\eta_p^2 = 0.029$). It is also worth noting that FIQ explained most of the variances in the model ($F_{(1,122)} = 137.400$, $p < 0.001$, $\eta_p^2 = 0.530$). Planned comparisons indicated that adult males with ASC performed worse than typical males ($p = 0.001$), which was not the case between the female groups ($p = 0.828$). Sex differences were observed in the control groups ($p < 0.001$) but not in the ASC groups ($p = 0.046$). See Figure 3-5, panel A. Note that similar to what was found in a previous study (White & Saldana, 2011), this all-item mean RT was highly correlated with accuracy (Spearman's $\rho = -0.93$, $p < 0.001$) so should be viewed as reflecting mainly information about accuracy, which itself was distributed far from normal and could not be analysed adequately with the current framework.

To explore purely the performance speed, a second ANCOVA was conducted for the *mean RT for correct items* only, with the same fixed factors and covariates but entered accuracy (log-transformation of the reflected accuracy, i.e., subtracted from 2,

to approximate normality) as an additional covariate to reduce the influence of different accuracy on performance speed (White & Saldana, 2011). Two men and one woman with ASC were excluded because they failed all 12 items. We noted a significant main effect of sex ($F_{(1,118)}$ = 4.063, p = 0.046, η_p^2 = 0.033) but not for diagnosis or their interaction. FIQ still explained most of the variances in the model ($F_{(1,118)}$ = 16.922, p < 0.001, η_p^2 = 0.126). Post-hoc comparisons showed non-significant sex differences in both typical control (p = 0.181) and ASC groups (p = 0.171). See Figure 3-5, panel B.

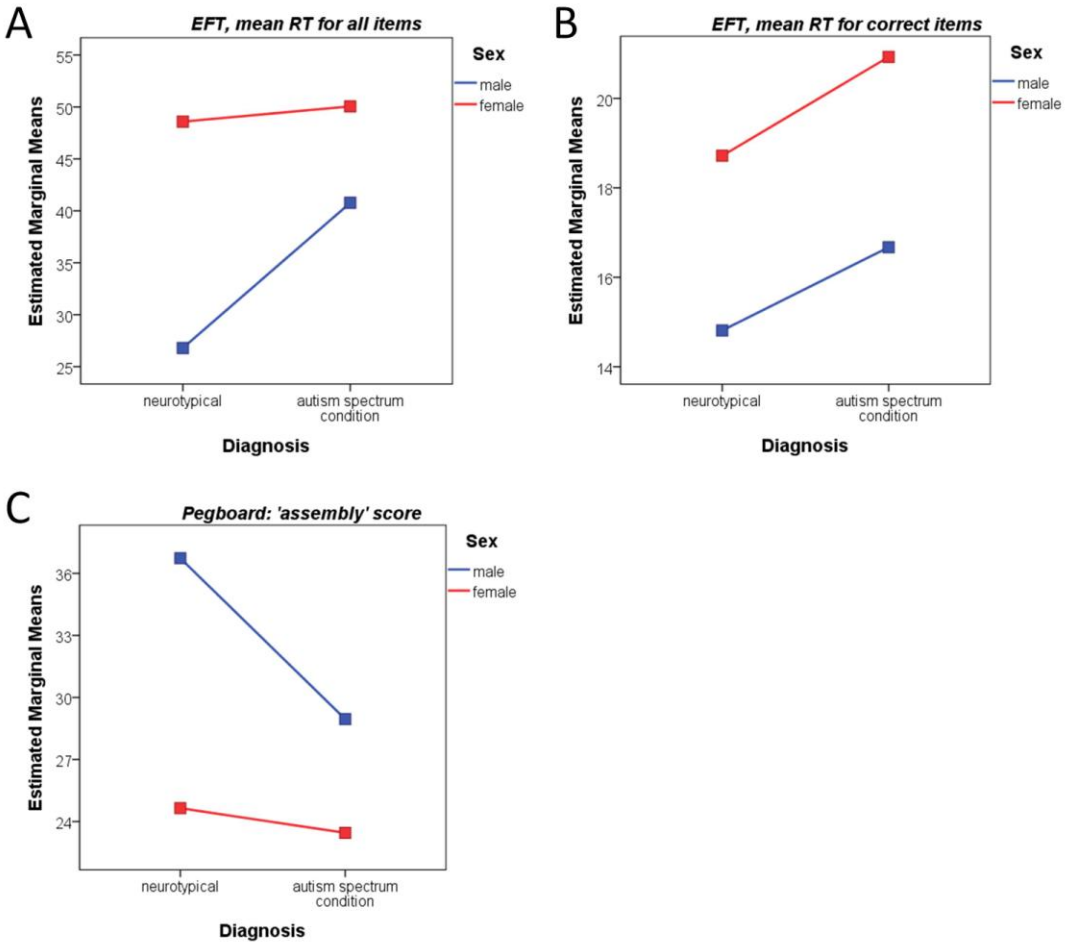
3.3.6 Dexterity

A MANCOVA treated the four outcome measures of the Purdue Pegboard Test (i.e., right, left, both hands scores and assembly score) as dependent variables, sex and diagnosis as fixed factors, and age and FIQ as covariates. There were significant main effects of sex (Pillai's Trace V = 0.459, $F_{(4,119)}$ = 25.229, p < 0.001, η_p^2 = 0.459), diagnosis (Pillai's Trace V = 0.218, $F_{(4,119)}$ = 8.280, p < 0.001, η_p^2 = 0.218) and sex-by-diagnosis interaction (Pillai's Trace V = 0.172, $F_{(4,119)}$ = 6.178, p < 0.001, η_p^2 = 0.172). Post-hoc ANCOVAs indicated that the significance was driven by the assembly score with significant effects of sex ($F_{(1,122)}$ = 70.815, p < 0.001, η_p^2 = 0.367), diagnosis ($F_{(1,122)}$ = 18.016, p < 0.001, η_p^2 = 0.129) and an interaction ($F_{(1,122)}$ = 9.895, p = 0.002, η_p^2 = 0.075). None of these were significant for the right, left or both hands scores. Planned comparisons indicated that for the assembly subtest, adult males with ASC performed worse than typical males (p < 0.001), which was not the case between the female groups (p = 0.200). A sex difference was observed in both the control (p < 0.001) and ASC groups (p < 0.001). See Figure 3-5, panel C.

We further repeated the ANCOVA on the assembly score by additionally entering either right or left hand subtest scores as a covariate to control for basic motor-speed difference. These did not change the pattern of group differences and significance.

Figure 3-5 EFT and Purdue Pegboard Test assembly performances of the four groups

Both EFT RT for all items (reflecting mainly accuracy, panel A) and assembly subtest score in the Purdue Pegboard Test (panel C) showed interaction between sex and diagnosis. Men with ASC performed worse than typical men, but women with ASC performed equally well as typical women. However EFT RT for correct items (reflecting purely processing speed, panel B) showed only a main effect of sex that altogether women were slower than men. Convention of the graphs is the same as that in Figure 3-1.

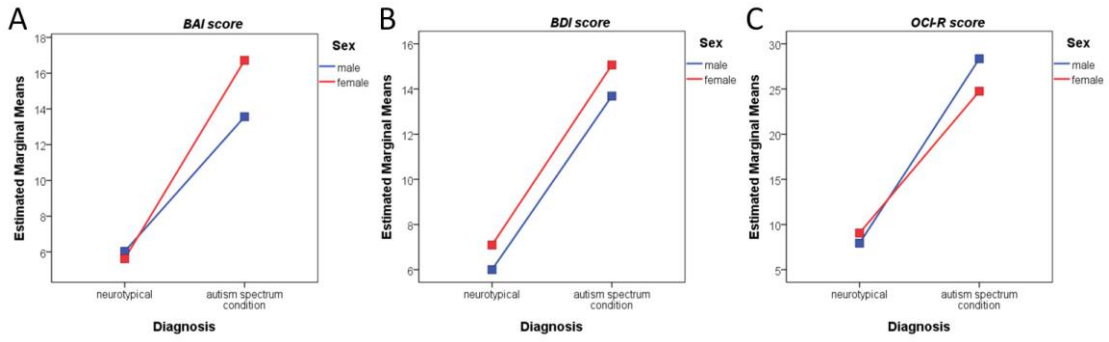


3.3.7 Co-occurring psychiatric symptoms

The last MANOVA treated the three measures of co-occurring psychiatric symptoms (i.e., BAI, BDI, OCI-R) as dependent variables, and sex and diagnosis as fixed factors. There was a significant main effect of diagnosis (Pillai's Trace V = 0.449, $F_{(3,122)}$ = 33.192, p < 0.001, η_p^2 = 0.449) but not of sex, nor was there a significant interaction. Post-hoc ANOVAs confirmed that the significant main effect of diagnosis was evident in all three measures: BAI ($F_{(1,124)}$ = 37.945, p < 0.001, η_p^2 = 0.234), BDI ($F_{(1,124)}$ = 31.556, p < 0.001, η_p^2 = 0.203) and OCI-R ($F_{(1,124)}$ = 93.777, p < 0.001, η_p^2 = 0.431). Planned comparisons confirmed the presence of significant simple effects of diagnosis across both sexes for all three measures, and no sex difference was noted in either ASC or control groups. See Figure 3-6.

Figure 3-6 Co-occurring psychiatric symptoms of the four groups

Irrespective of sex, adults with ASC reported significantly more severe symptoms on BAI (panel A), BDI (panel B) and OCI-R (panel C). Convention of the graphs is the same as that in Figure 3-1.



3.4 Discussion

Do cognitive characteristics of ASC vary with sex? The answer is yes and no, *depending on the domain of functioning under examination*. This ambiguity points to the complex relationship between sex and ASC, in regards to the heterogeneous cognitive presentations of ASC. The findings reported in Chapters 2 and 3, however, have pushed current understandings a step further in illuminating the heterogeneity by pinpointing the similarities and differences between males and females with ASC, as well as clarifying how sex modulates ASC presentations in the key domains.

3.4.1 Domain-dependent sex-ASC relationship

3.4.1.1 Dispositional traits

In accordance with previous empirical findings (Goldenfeld, et al., 2005; Wheelwright, et al., 2006) and the predictions from the EMB theory (Baron-Cohen, 2002, 2010; Baron-Cohen, Knickmeyer, et al., 2005), we found typical sex differences in dispositional empathy, systemizing and autistic traits (i.e., the first characteristic for an EMB pattern), which was attenuated in ASC (i.e., the second characteristic for an EMB pattern). This attenuation was so obvious to result in a statistically significant sex-by-diagnosis *ordinal* interaction. This could meanwhile be interpreted as a much stronger ASC effect on these traits in females than in males (hence the typical sex difference disappeared in ASC). For AQ this effect even reversed the typical sex difference that women with ASC scored higher than men with ASC, for potential reasons discussed in Chapter 2. On TAS there was a significant main effect of diagnosis only, but no typical sex difference or sex-by-diagnosis interaction,

indicating across-sex difficulties in understanding and expressing own emotions for adults with ASC. The sex-ASC relationship in the domain of dispositional traits could thus be considered generally fitting with the EMB model up to fulfilling an ordinal interaction (see Chapter 1). TAS instead fit with the OG model.

3.4.1.2 Mentalizing and emotion perception

Cognitive measures in the domain of mentalizing and basic facial emotion perception showed a pattern similar to that of alexithymia which did not match with the EMB model, rather there was simply a main effect of ASC. Regardless of sex, as long as one had ASC her/his abilities in mentalizing and basic facial emotion recognition were impaired. This fits the orthogonal (OG) model (see Chapter 1). It is not surprising given this domain is core to how ASC is defined. Our sample selection conservatively included only those with evident childhood autistic symptoms. Therefore, male and female adults with ASC here are likely to be those who suffer from similarly severe levels of core socio-communication difficulties, reflected here by the corresponding social cognitive weakness. On the other hand, the sex-similarity within ASC regarding poor social cognitive abilities, together with (and in sharp contrast to) the sex-difference in interactive behaviours revealed by the ADOS (i.e., milder autistic features in women compared to men with ASC), strengthen the argument made in Chapter 2 about the plausible camouflage and superficially intact socio-communication abilities in women with ASC.

The advanced mentalizing deficit in adults with ASC replicates a wealth of previous studies (Baron-Cohen, Wheelwright, Hill, et al., 2001; Golan & Baron-Cohen, 2006; Lombardo, et al., 2007; Losh et al., 2009). For basic facial emotion recognition, adults with ASC in the present study showed extensive

difficulties in recognising emotion faces across all basic emotions, in contrast to previous studies which often found impairments on negative emotions but spared positive ones (Ashwin, Chapman, et al., 2006; Corden, et al., 2008; Howard, et al., 2000; Humphreys, et al., 2007; Law Smith, et al., 2010; Wallace, et al., 2011). Two potential reasons may contribute to this, other than basic emotion recognition ability *per se*: a generally slower stimulus-reaction response, and/or difficulties in processing face. We unfortunately did not have a measure of simple stimulus-reaction response (e.g. RT on a simple and neutral stimulus-response task) so were not able to directly rule-out potential confounds from a generally slower stimulus-reaction. On the closest measure we have – the RT for the "go" responses on the Go/No-Go task – people with ASC indeed performed slower, and this RT was moderately correlated with all KDEF Test RTs. We therefore tried to tease this general slowness apart by correcting each emotion RT by this go-response RT for individual participants, and the group comparison results remained the same with a significant main effect of diagnosis across all emotions (happy: $p < 0.001$, sad: $p = 0.001$, angry: $p = 0.014$, fear: $p < 0.001$, disgusted: $p = 0.004$, surprised: $p = 0.001$, neutral: $p = 0.009$). This additional test suggests that even after controlling for generally slower stimulus-reaction response, adults with ASC were still slower in recognising all basic emotion faces than typical adults.

On the other hand, to what extent this extensive impairment is accounted for by difficulties in face processing is unclear given the design of the task. We suspect the influence is substantial given repeated reports on face processing difficulties in people with ASC (Davis & Plaisted, 2007; Golarai, Grill-Spector, & Reiss, 2006; Hernandez et al., 2009; O'Hearn, Schroer, Minshew, & Luna, 2010; Sasson, 2006; Schultz, 2005;

Schultz, et al., 2000; Schultz, et al., 2003) which may interfere with facial emotion processing. However recent report also shows independent facial identity versus facial emotion processing in children with ASC (Krebs et al., 2011). Whether atypical facial emotion processing in ASC is primary or secondary to other social deficits (e.g. face processing difficulties, low interest in social interaction and/or avoidance to scan other's eyes region) in ASC awaits investigation (Harms, et al., 2010).

For specific emotions, we did find that adults with ASC were especially poorer than typical adults in identifying fear compared to happy or neutral faces. This is in line with previous reports on the particular difficulty in processing fear for people with ASC (Ashwin, Chapman, et al., 2006; Corden, et al., 2008; Howard, et al., 2000; Humphreys, et al., 2007) and further demonstrates that it is sex-independent.

3.4.1.3 Executive function

Regarding executive dysfunction, the sex-ASC relationship is further dependent on the sub-domains. We found reduced sensitivity to signal (also evident by more omission errors) in both sexes of adults with ASC but the impairment was weaker for females. We also noted a weak diagnostic group difference in simple response inhibition (reflected by commission error). The diagnosis did not make a difference on phonological memory and generativity (i.e., verbal fluency). However, it made a clear difference on the dexterity subtest of assembly which required certain extent of motor coordination, inhibition and planning on top of basic motor speed, where men with ASC were impaired compared to typical men but women with or without ASC performed similarly. This difference is hard to be accounted for purely by gross or fine motor deficits since a diagnostic group difference was not observed in any of the three simpler dexterity subtests. In addition, even after controlling for basic motor

speed the group difference pattern in assembly subtest performance remained the same. In sum, we replicated the lack of ASC diagnostic differences on (phonological) working memory and (verbal) generativity (Boucher, 2009; Hill, 2004a, 2004b), but found a strong *sex-specific effect* (SSE, see Chapter 1) on dexterity subtest involving motor executive function. We also noted an across-sex deficit (but stronger in males) on sensitivity to signal, and a weak deficit on simple response inhibition for adults with ASC.

SSE for ASC in executive function has recently been reported in children and adolescents. Adolescent females with ASC outperformed males with ASC on the Trail Making Test (for set-shifting) but the opposite was observed in unaffected sibling controls (Bolte, et al., 2011). Girls (aged 6 to 16) with ASC performed worse than typical girls on a stop signal task (for response inhibition) in terms of longer stop signal reaction time, but boys with ASC performed comparably as control boys and girls, who did similarly well (Lemon, et al., 2011). For executive function, performances in different ages are difficult to compare due to developmental changes. However, our findings of SSE on motor executive function related to dexterity (and potentially but non-significantly on signal sensitivity) add evidence to support sex-dependent features in executive function in ASC and highlight the need for further systematic investigation.

The impaired sensitivity to signal yet comparable response strategy in ASC are of additional interest. Both indexes were calculated via SDT, a well-validated theoretical framework for human perception and judgment. However the observation here was solely based on the Go/No-Go task performance. Whether the impaired sensitivity involves other sub-domains of executive function or an even broader range

of cognition is critical in identifying its specificity. If it stands out in the majority of cognitive performances, a general bottom-up attention bias/deficit should be considered fundamental to ASC and might substantially inform or revise the current cognitive theories for autism.

Our ASC participants performed similarly well as typical adults in two language-related executive functions, i.e., phonological working memory and verbal generativity. The former has been reported to be impaired in adolescents with ASC with language impairment (Loucas et al., 2010) and the latter inconsistently reported to be impaired in ASC (Hill, 2004a; Turner, 1999). Our null finding may result from sample characteristics. The ASC groups in this study were consisted of mainly high-functioning adults without current language impairments, so their executive functions serving basic language abilities were likely unimpaired. This again marked the great heterogeneity within ASC.

3.4.1.4 Perceptual attention to detail

It has been pointed out that reports were markedly inconsistent on EFT performance in ASC – better, similar or even worse than controls. This is possibly due to different methodological and analytical strategies as well as heterogeneity in central coherence within ASC (White & Saldana, 2011). On both outcome measures, i.e., mean RT for all items (reflecting mainly accuracy) and mean RT for correct items adjusted by accuracy (reflecting processing speed), we replicated the typical male superiority (Voyer, et al., 1995). Furthermore in the ASC group, the trend level significance ($p < 0.046$) of male superiority on the mean RT for all items corresponds to previous reports of better performance in males than females with ASC on the Block Design task (which also relies on visuospatial attention to detail) in children

(Koyama, et al., 2009) and adolescents (Bolte, et al., 2011). However, we did not replicate the ASC superiority noted by some early studies (Jolliffe & Baron-Cohen, 1997; Shah & Frith, 1983). Rather the results correspond better with the recent well-powered study in children showing no such superiority on EFT performance (White & Saldana, 2011).

For RT for all items, adults with ASC performed poorer than typical adults only in males but not in females. This SSE (in accuracy) suggests that perceptual attention to detail characterises men with ASC from those without, but it is unrelated to the cognitive features of women with ASC. The lack of superiority (or even worse) in ASC also challenges the predictions from the WCC theory. However, whether EFT really disentangles global from local processing is unclear, so interpretations should be made cautiously (White & Saldana, 2011).

3.4.1.5 Dexterity

The lack of group difference in simple dexterity subtests (i.e., right hand, left hand and both hands alternatively) failed to suggest a general motor clumsiness in our sample of adults with ASC, in contrast to previous findings (Fournier, et al., 2010). This is likely due to the fact that we recruited high-functioning adults and hardly any of them had a history of motor delay. Neurological comorbidities, including motor clumsiness, are usually associated with the more severe behavioural phenotype in ASC (Jeste, 2011). Hence it is plausible that these high-functioning adults escape from motor impairments.

However, we did find an SSE for the assembly subtest. As illustrated earlier it involves certain extent of motor coordination, planning and inhibition in addition to

basic gross and fine motor skills. A stand-alone poor performance in assembly but not simpler subtests may reflect motor executive dysfunction involving dexterity. This was found, however, only in males but not in females with ASC. This SSE warrants further investigation on whether motor executive dysfunction is specific to males but not females with ASC.

We did not find a female advantage on the assembly subtest in typical controls as reported before (Nicholson & Kimura, 1996), even when controlling for basic motor speed (reflected by right or left hand scores). The reason for this non-replication is unknown (perhaps due to different sample characteristics) and awaits validation.

3.4.1.6 Co-occurring psychiatric symptoms

Besides marking the clinically significant co-occurring anxiety, depression and obsessive-compulsive symptoms for adults with ASC as reported in Chapter 2, this chapter further highlighted their strikingly more severe symptoms compared to typical adults, irrespective of sex. We did not replicate previous reports of higher severity in females than in males with ASC on internalising symptoms in adolescents (Solomon, et al., 2011) or on total behavioural problems in children (Holtmann, et al., 2007). However, we have replicated the sex-similarity in co-occurring neurotic symptoms in adults noted by other independent clinical studies (Hofvander, et al., 2009; Lugnegard, et al., 2011). Whether the discrepancy is due to differences in age or other demographic or cognitive characteristics awaits future investigation, especially by utilising longitudinal follow-up designs.

3.4.2 Central-peripheral discrepancy of sex-ASC relationship in cognition?

Each cognitive domain discussed above was investigated by only a limited number of tasks. Therefore a cross-domain summary and inference must be preliminary and speculative. However from findings in this and the previous chapters, we have observed a plausible pattern of the relationships between sex and ASC across domains, which may inform future investigations.

For behaviours that define ASC (i.e., childhood behaviours and developmental history) males and females showed comparable symptom severity. This can be viewed as a strong ASC effect across sex (since typical adults will by definition score zero or very low on the ADI-R), meanwhile fitting the OG model. In adulthood, dispositional traits reflected the core feature of ASC, which also showed a strong ASC effect across sex. Although there was an interaction between sex and diagnosis on these traits, it was a very special case (i.e., an *ordinal* interaction) that rendered the pattern to fit the EMB model. Similarly for another central feature related to ASC, namely mentalizing and emotion perception, there was also a strong ASC effect independent of sex, again fitting the OG model. It is thus plausible that for these *defining central features*, males and females with ASC are similar in being different from their respective typical counterparts (thus fitting the OG model), or that there is even a stronger difference in females (and fitting the EMB model).

When it comes to the more *peripheral* domains (i.e., not directly related to defining or diagnosing people as having ASC) the pattern seems to be different. For perceptual attention to detail, executive function, dexterity and current interactive behaviours (which could have been modified substantially during development), as

long as there is a diagnostic group difference in performance, it is usually with a *disordinal* sex-by-ASC interaction (thus fitting the SSE model) that the ASC effect is more prominent in males than in females. This *central-peripheral discrepancy of sex-ASC relationship in cognition* marks one potential route to disentangle the heterogeneity of ASC by sex and may help guide future research designs. One example of this will be considering the stratification by sex as necessary.

3.4.3 Limitations and future directions

Findings in this chapter are derived from multiple tasks across cognitive domains, yet are still liable to certain limitations. First, not all cognitive domains related to ASC in adults were examined. For example, we did not assess advanced language function particularly in semantics and pragmatics, which are likely to be atypical in high-functioning adults with ASC. Sub-domains of executive function most consistently reported to be impaired in ASC (i.e., set-shifting and planning) were also not specifically examined. Second, although the chosen tasks are classical and have been applied in autism research for decades, they may not be the best to address the cognitive theories for autism in certain domains. For example, perhaps a Navon task may better address global-local attention bias (Plaisted, Swettenham, & Rees, 1999) than the EFT, and spontaneous ToM (Senju, 2011) or moral judgment ToM tasks (Moran, et al., 2011) may further inform fine-grained aspects of mentalizing abilities in high-functioning adults. Third, to fully address the relationship between sex and ASC, we should have also included tasks/domains showing the largest effect sizes of typical sex differences, such as visuospatial abilities of targeting and mental rotation or aggression tendency (Hines, 2010; Voyer, et al., 1995). To overcome these

limitations and to extend the study in the future, it is best to utilise a more comprehensive battery as what is done by other ongoing studies (Charman, et al., 2011).

Fourth, this chapter only illustrated straightforward comparisons across the four groups on the conventional outcome measures of each task, with the *a priori* interest in exploring sex-ASC relationship for each cognitive domain. Owing to the complex cognitive processes involved in individual tasks and the intermingled relations amongst task-common and task-specific cognitive abilities, advanced analytical methods may be capable of illuminating the *latent structure* and how it is affected by sex and ASC. Strategies such as factor analysis on all items of the dispositional traits, examining sensitivity to signal and response strategy across tasks by the SDT framework, or multivariate approaches (e.g., discriminant analysis or classifier algorithm) may all be informative. Furthermore, for certain tasks, more refined analyses may help discover phenomena not observed by current analytical strategies, for example to analyse how different emotions are mis-recognised as others (i.e., the 'confusion matrix' from the KDEF Test).

Lastly, the male and female groups were recruited and tested in two different periods. We ensured all task materials and procedures to be exactly the same, and for the ADI-R and ADOS we had a senior trainer (Dr. Greg Pasco) involved in both periods to ensure reliability. However the cognitive assessments for the male and female groups were conducted by two different teams of researchers (for males, Dr. Lombardo and colleagues; for females, myself and colleagues). How this might affect the variance structure is unknown. If it does have an effect it is unfortunately inseparable from the variance explained by sex.

Chapter 4

Neuroanatomical Features of Female Adults with ASC:

Voxel-Based Morphometry

4.1 Introduction

The previous chapters have demonstrated that in behaviour and cognition, there are similarities and differences between male and female adults with high functioning ASC compared to typical controls. To further explore the brain basis of ASC, one straightforward way is to examine the morphology of the brain. Given technical progress of neuroimaging, it is now possible to perform *in vivo* brain morphometry through structural magnetic resonance imaging (MRI).

Whilst there are consistent differences in behaviour and cognition between people with and without ASC, neuroanatomical differences are strikingly *inconsistent* (Brambilla et al., 2003; Stanfield et al., 2008), which may be due to different factors such as the heterogeneity of ASC (e.g. differences related to age, IQ, sex, co-occurring conditions or history of language development) and variability in analytic methods. For example, different analytic methods yield only partially overlapping findings. A recent qualitative meta-analysis (R. Chen, Jiao, & Herskovits, 2011) concludes that region-of-interest (ROI)-based analysis shows reduced corpus callosum and increased amygdala volume (the latter only in children) in people with ASC. Voxel-based morphometry (VBM) shows no consistent pattern of regional specificity when density-based and volume-based analyses are pooled together, but separately people with ASC show increased grey matter (GM) volume but decreased density in frontal and temporal lobes, and increased white matter (WM) volume and density in the temporal lobe. Surface-based morphometry (SBM) yields inconsistent directions of change in cortical thickness (sparing occipital lobe), but the majority report an increase in the parietal lobe. Diffusion tensor imaging studies relatively

consistently report abnormalities from childhood to adulthood in the corpus callosum, internal capsule, prefrontal and cingulate WM.

VBM, a mass-univariate non-biased approach to demonstrate volumetric differences between groups across the whole brain (Ashburner & Friston, 2000), is most frequently applied. From the first VBM study on autism (Abell et al., 1999), over 20 studies have reported GM volumetric differences in a variety of brain regions, including cerebellum, limbic system (e.g. amygdala-hippocampal complex), basal ganglion, hypothalamus, thalamus and a range of fronto-temporal structures in childhood/adolescent (Boddaert et al., 2004; Bonilha et al., 2008; Brieber et al., 2007; Cheng, Chou, Fan, & Lin, 2011; Freitag et al., 2008; Ke et al., 2008; Kurth et al., 2011; Kwon, Ow, Pedatella, Lotspeich, & Reiss, 2004; Langen et al., 2009; McAlonan et al., 2008; Waiter et al., 2004) and adult populations (Abell, et al., 1999; M. C. Craig et al., 2007; Ecker, Rocha-Rego, et al., 2010; K. L. Hyde, Samson, Evans, & Mottron, 2010; Kosaka et al., 2010; McAlonan et al., 2002; Rojas et al., 2006; Schmitz et al., 2006; Toal et al., 2010; Wilson, Tregellas, Hagerman, Rogers, & Rojas, 2009), yet with limited overlap among studies. Similarly, over 10 studies have examined WM volumetric differences and yielded inconsistent group differences in a variety of regions (Boddaert, et al., 2004; Bonilha, et al., 2008; M. C. Craig, et al., 2007; Ecker, Rocha-Rego, et al., 2010; K. L. Hyde, et al., 2010; Ke, et al., 2008; Ke et al., 2009; McAlonan et al., 2009; McAlonan, et al., 2002; Toal, et al., 2010; Waiter et al., 2005). Owing to the marked inconsistency, a more solid picture has emerged from recent quantitative meta-analyses which have included the largest sample to date using Signed Differential Mapping (SDM). These quantitative meta-analyses conclude that for GM, people with ASC have less volume in bilateral

amygdala-hippocampal complex and precuneus, and increased volume in a region at left middle inferior frontal gyrus, compared to typical controls (Via, Radua, Cardoner, Happe, & Mataix-Cols, 2011). With regards to WM, people with ASC show increased volume at the anterior portion of the right arcuate fasciculus, and the anterior portion of the left inferior fronto-occipital fasciculus along with the superior portion of the left uncinate fasciculus (Radua, Via, Catani, & Mataix-Cols, 2011).

One reason for such inconsistency may be the lack of power of the previous designs for detecting neuroanatomical differences between people with and without ASC. For a given significance criterion, a lack of power results from insufficient sample size and/or low effect size for group difference. It has been estimated that in a multi-centre study design for male adults, controlling for false discovery rate at q = 0.05, it requires a minimal sample size (for each group) of 15 to detect group differences in inferior frontal regions, but for subcortical regions it requires more (i.e., up to >30 for caudate and >70 for amygdala) (Suckling et al., 2010). For the majority of the VBM studies to date the sample size is less than 20 and this may have significantly compromised the power to detect group differences and contribute to the inconsistency in the literature. One attempt to significantly increase sample size in studying neuroanatomical features of adult males with ASC has been conducted by the MRC AIMS consortium in conjunction with studies carried out in this dissertation (Ecker et al., in press).

On the other hand, the marked heterogeneity in ASC may significantly compromise the effect size for group differences, reducing the effect size encountered if more homogeneous subgroups were examined. Apart from known factors contributing to the heterogeneity of brain anatomy in ASC such as age (Courchesne,

Campbell, & Solso, 2011; Raznahan, Toro, et al., 2010) and IQ (Lotspeich, et al., 2004), *sex* is a factor that is nearly always overlooked. If, as suggested in Chapter 1, sex and ASC affects brain anatomy in a non-orthogonal manner, then examining the main effect of ASC by grouping the sexes together simply increases sample heterogeneity, thereby reducing the effect size even when the groups are matched on sex. All but one VBM studies to date examine either all-male or mixed but predominantly male (>80%) sample, from which the effect of sex cannot be examined, and for the mixed sample studies participant heterogeneity may be particularly large. Investigating males and females separately will not only help clarify the complex relationship between sex and ASC (reported in Chapters 5 and 6), but is also likely to increase the effect size of group difference by reducing heterogeneity. In the instance where stratifying the sample by sex increases effect size by reducing heterogeneity, this would raise the larger question about whether there are sex-specific neural biomarkers for ASC (Schwarz, et al., 2010).

There are only three studies to date contrasting brain anatomy of females with and without ASC (Bloss & Courchesne, 2007; M. C. Craig, et al., 2007; Schumann, et al., 2010), and only one of them has used VBM (M. C. Craig, et al., 2007). Craig et al. compare 14 women with ASC (mean age 37.9 years, standard deviation, SD = 11.4) and 19 typical women (mean age 35.0 years, SD = 14.0) showing that women with ASC have smaller GM volume in right anterior cingulate, bilateral basal ganglia and diffuse lateral and medial temporal structures; they also have smaller WM volume at medulla oblongata, pons and temporal area, but show a diffuse increase in WM volume within frontal and posterior temporal and parietal structures. The regions discovered, however, only partially overlap with what have been summarised by the

meta-analyses on predominantly male samples.

This chapter reports a VBM study with the largest sample size to date for high-functioning female adults with and without ASC, to elucidate their neuroanatomical features. This will serve as the basis for further investigation on the relationship between sex and ASC in terms of brain anatomy, addressed in Chapter 5.

4.2 Methods

4.2.1 Participants

Thirty-eight right-handed female adults with a formal clinical diagnosis of ASC (i.e., Asperger syndrome or autistic disorder according to the DSM-IV or ICD-10 criteria, diagnosed by experienced chartered psychiatrist or clinical psychologist working in the UK National Health Service), and 35 female adult control participants were recruited, by advertisements in the community in conjunction with the UK MRC Autism Imaging Multicentre Study (AIMS) project. See Chapters 2 and 3 for recruitment details. Exclusion criteria for both groups included psychotic disorders, substance-use disorders, severe head injury, syndromic genetic disorders associated with autism (e.g. fragile X syndrome, tuberous sclerosis), intellectual disability (i.e. IQ < 70), hyperkinetic disorder, Tourette's disorder or any other medical condition significantly affecting brain function (e.g. epilepsy). We however included people who had an isolated seizure as children, if they were not treated for longer than 6 months, and people with a history of mood, anxiety or obsessive compulsive disorder as these are commonly associated with adults with ASC (Hofvander, et al., 2009; Lugnegard, et al., 2011; Tantam, 2000). To be eligible for MRI scanning, participants

also need to meet the criteria of not having metal implants/objects in their body, not being pregnant and not having claustrophobia. The inclusion criteria for the control group additionally required not having ASC themselves nor in their family history. All participants gave informed written consent in accordance with the ethics approval from the National Research Ethics Committee, Suffolk, UK.

Among the 38 women with ASC, four were excluded due to sub-threshold ADI-R scores by the same criteria described in Chapter 2, and another four excluded due to motion artefact that resulted in unsatisfactory image quality. In the remaining 30 participants, two did not have ADI-R available (their childhood caregivers were not able to provide interviews). For these two participants, one scored above the cut-off for 'autism spectrum' on the ADOS and the other was positive for a diagnosis on the Adult Asperger Assessment (AAA) (Baron-Cohen, Wheelwright, et al., 2005) which incorporates care-giver reports of childhood behaviours and developmental history. Thus both participants were included for analysis, leaving a final sample of 30 ASC participants. For the 35 control participants, one did not finish the scanning session due to claustrophobia and two other participants were excluded due to motion artefact. From the remaining 32 control females, we selected 30 to match with the ASC group in terms of age, verbal, performance and full-scale IQ. This resulted in a matched sample of 30 participants per group for analyses.

4.2.2 Behavioural assessments

All participants were assessed using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) which measures verbal, performance and full-scale IQ. All ASC participants were assessed by the ADI-R and ADOS, along

with a series of self-report and neuropsychological assessments (details given in Chapters 2 and 3).

4.2.3 Structural MRI data acquisition and processing

All participants were scanned using a contemporary 3T MRI scanner (GE Medical Systems HDx) fitted with an 8-channel receive–only RT head-coil at the Magnetic Resonance Imaging and Spectroscopy Unit (MRIS Unit), Department of Radiology, University of Cambridge, between November 2009 and October 2010. A set of clinical structural MRI scans were acquired and a radiologist screened each scan to exclude clinically significant abnormalities. For the present study, a specialised acquisition protocol employing quantitative imaging – Driven Equilibrium Single Pulse Estimation of T_1 (DESPOT1) – was utilised since it was part of multi-centre imaging projects (the MRC AIMS and the Female AIMS projects) which required standardisation of structural MRI scans across the three scanner platforms at the Cambridge, London and Oxford sites. This protocol has previously been validated and is described extensively elsewhere (Deoni et al., 2008). In short, spoiled gradient recalled (SPGR) were acquired at two flip angles (α) from which an estimate of the absolute T_1 value was derived at each voxel. These quantitative T_1 maps were then used to create simulated structural T_1-weighted inversion recovery (IR) images, with 176 contiguous slices (1 mm x 1 mm x 1 mm resolution), a field-of-view of 25.6cm, a simulated repetition time/inversion time (TR/TI) of 1800/850ms, a scaling constant ρ = 10000 and a flip angle of 20°. This combination of parameters gave excellent deep and cortical grey/white matter contrast in the subsequent tissue segmentation without the need of modulation by B_0 and B_1 field inhomogeneities because compensation had

been introduced during the estimation of absolute T_1.

The simulated T_1-weighted IR images were then segmented and normalised to the standard MNI space using the SPM8 software (Wellcome Department of Imaging Neuroscience Group, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Native-space grey matter (GM), white matter (WM) and cerebral-spinal fluid (CSF) images were obtained using standard automated segmentation routines in SPM8. Individual total GM, WM and CSF volumes were estimated by summing up the partial volume estimate throughout each class of segmented image in the native space, and total brain volume (TBV) was estimated by summing up GM and WM volumes. The native-space GM and WM images were then registered to a study-specific template generated from all 60 female participants using a high-dimensional non-linear diffeomorphic registration algorithm (DARTEL) (Ashburner, 2007). This non-linear warping technique minimises inter-individual structural variance and thereby improves the sensitivity of VBM analysis. A modulation step was included to retain voxel-wise information about local tissue volume. After mapping to standard space, the resulting modulated GM and WM maps were smoothed with a 4mm full-width-half-maximum (FWHM) Gaussian kernel. A 4mm smoothing kernel was chosen to retain finer local information because (i) the original scans were obtained in high spatial resolution (1mm isotropic), (ii) DARTEL provided more accurate registration than conventional normalisation methods, (iii) the modulation provided extra smoothness already, and (iv) 4mm extra smoothing has been shown to provide adequate specificity in VBM for images registered through DARTEL (Henley et al., 2010).

4.2.4 Statistical analysis

Voxel-wise statistical testing on the modulated GM and WM images was performed with SPM8. To avoid possible edge effects between different tissue types, the GM group comparison was constrained within the GM segment of the study-specific template image generated by DARTEL with a threshold of voxel value (i.e., partial volume estimate) above 0.25; a parallel procedure was introduced for WM group comparison. Furthermore, taking advantage of modulation, information of both local tissue volume and total volume was retained. Prior to statistical modelling, each modulated GM map was individually re-scaled to its own global mean (which reflected its own total GM volume) to derive a map indicating *relative local GM volume (i.e., corrected for total GM volume)*. A parallel procedure for WM was also conducted to generate individual maps of *relative local WM volume (i.e., corrected for total WM volume)*. The aims and benefits of this "proportional adjustment/scaling" procedure (O'Brien et al., 2006) were based on three justifications. (i) We are interested in relative instead of absolute local volume. (ii) By individual level adjustment, it is not necessary to include a total GM/WM measurement as a covariate in the statistical model and thus, saving one degree of freedom and increasing the stability of the model. (iii) If there is a significant difference in total volume between groups, entering total volume as a covariate in the model will reduce the power to detect group differences because this covariate is correlated with the independent variable of interest (i.e., group) (Suckling, 2011). Adjustment at the individual level will bypass this problem to compare relative local volume between groups.

We then regressed the general linear model (ANCOVA) at each voxel where the diagnostic group was the fixed factor, and age (linear term) was the nuisance

covariate to remove variance in brain volume explained by age. Statistical outcomes were corrected for multiple comparisons by controlling topological false discovery rate (FDR) calculated under Gaussian Random Field Theory (Chumbley & Friston, 2009). Here we used a cluster-forming height threshold of $p < 0.025$ for each contrast and a spatial extent threshold that ensures a FDR at $q < 0.05$ for clusters.

Independent sample *t*-tests were used to compare behavioural and global brain volumetric measures, and Pearson's correlations were used to explore the relationship between relative volumes (of the brain regions showing significant group differences) and behavioural measures. These analyses were performed with the PASW Statistics version 18 (SPSS Inc., Chicago, IL, USA).

4.3 Results

4.3.1 Behavioural characteristics

The typical control (NT, stands for "neurotypical") and ASC groups were matched on age, VIQ, PIQ and FIQ with all group differences non-significant ($p > 0.05$). As predicted, and described in Chapter 3, the ASC group scored significantly higher on the Autism Spectrum Quotient (AQ), revised Systemizing Quotient (SQ), and lower on the Empathy Quotient (EQ) than the NT group with large effect sizes. See Table 4-1 for details.

Table 4-1 Behavioural characteristics for female adults with and without ASC

	NT (N=30) Mean (SD)	ASC (N=30) Mean (SD) [range] [$]	Statistics *t*	*p*	ES *d*
Age (Years)	27.5 (6.5)	27.8 (7.6)	-0.188	0.852	0.04
Verbal IQ	118.5 (9.6)	115.8 (13.1)	0.890	0.377	0.24
Performance IQ *	117.0 (9.3)	110.4 (16.7)	1.895	0.064	0.49
Full IQ *	120.2 (8.0)	114.9 (13.8)	1.799	0.078	0.47
AQ	12.0 (4.8)	37.5 (6.7)	-16.901	< 0.001	4.38
EQ	53.5 (9.5)	19.5 (7.5)	15.422	< 0.001	3.97
SQ *	47.0 (18.3)	73.5 (29.3)	-4.191	< 0.001	1.08
ADI-R [#]					
Social reciprocity	–	16.4 (4.3) [11 – 26]	–	–	–
Communication	–	13.1 (3.9) [8 – 22]	–	–	–
RSB	–	4.3 (1.7) [2 – 8]	–	–	–
ADOS					
S + C	–	4.3 (3.6) [0 – 13]	–	–	–
RSB	–	0.1 (0.3) [0 – 1]	–	–	–

[$]: For ADI-R and ADOS scores.

*: Levene's Test for Equality of Variances showed significant non-equal variances, therefore equal variance was not assumed in the *t*-tests.

[#]: N = 28.

NT = (neuro)typical control group female adults; ASC = female adults with ASC; ES: effect size; *d* = Cohen's *d*; SD = standard deviation; AQ = Autism Spectrum Quotient; EQ = Empathy Quotient; SQ = revised Systemizing Quotient; ADI-R = Autism Diagnostic Interview-Revised; RSB: repetitive, restrictive and stereotyped behaviour; ADOS: Autism Diagnostic Observation Schedule; S + C: ADOS "social interaction + communication" total scores.

4.3.2 Global volume difference

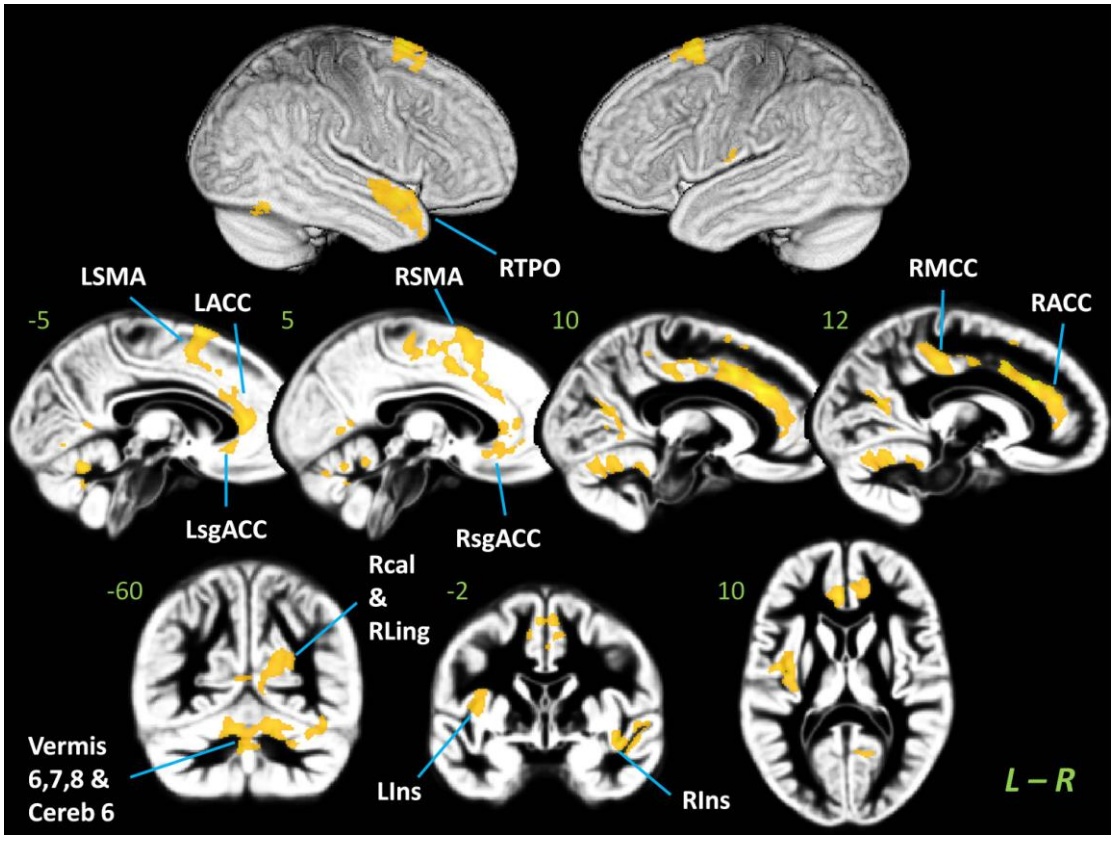
There was no group difference in estimated total GM volume (NT: 824 cm^3, SD = 81 cm^3; ASC: 845 cm^3, SD = 72 cm^3; t = -1.091, p = 0.280), total WM volume (NT: 448 cm^3, SD = 53 cm^3; ASC: 465 cm^3, SD = 47 cm^3; t = -1.316, p = 0.193) and total CSF volume (NT: 236 cm^3, SD = 53 cm^3; ASC: 227 cm^3, SD = 45 cm^3; t = 0.740, p = 0.462).

4.3.3 Regional volumetric difference in GM

The final effective smoothness for the 4mm FWHM kernel-smoothed modulated GM images was 7.2mm x 7.0mm x 7.0mm, which was sufficient for statistical inferences based on the Gaussian Random Field Theory to be valid given that it was more than three times the voxel size (1mm isotropic). Five clusters had relative regional volumes that were significantly larger in the NT compared to the ASC group. Cluster 1 (cluster size k_e = 14123, peak voxel T = 4.85, MNI coordinate [12,26,27]) spread across bilateral subgenual, anterior and middle cingulate cortices, and supplementary motor areas; cluster 2 (k_e = 2588, peak voxel T = 4.15, MNI coordinate [2,-65,2]) was located in right lingual and calcarine gyri close to midline; cluster 3 (k_e = 2682, peak voxel T = 3.94, MNI coordinate [-30,-14,17]) was located in left insula; cluster 4 (k_e = 5254, peak voxel T = 3.70, MNI coordinate [-4,-60,-28]) spread across cerebellar vermis lobule 6, 7, 8 and left and right cerebellar hemisphere lobule 6; cluster 5 (k_e = 3076, peak voxel T = 3.59, MNI coordinate [50,7,-9]) spread across right temporal pole and superior temporal gyrus extending inward to right insula; see Figure 4-1. There were no clusters showing greater relative volume in the ASC than in the NT group survived multiple comparison correction.

Figure 4-1 Smaller relative regional GM volume in women with ASC

Female adults with ASC had less relative regional GM volume in the clusters marked in orange, compared to typical female adults. Significant clusters were overlaid on the GM segment of the study-specific template image generated by DARTEL. The top row shows the lateral surfaces of the brain. Middle row (sagittal views, left to right) shows involvement of cortical and cerebellar midline structures; number in green at the left-upper corner of each slice indicates MNI coordinate on the x-axis. Bottom row shows coronal (left and middle) and axial (right) views in neurological convention (i.e., left is left and right is right); numbers in green indicate MNI coordinates on the y-axis (for coronal view) and z-axis (for axial view). LACC = left anterior cingulate cortex; LIns = left insula; LsgACC = left subgenual ACC; LSMA = left supplementary motor area; RACC = right anterior cingulate cortex; RCal & RLing = right calcarine and lingual gyri; RIns = right insula; RMCC = right middle cingulate cortex; RsgACC = right subgenual ACC; RSMA = right supplementary motor area; RTPO = right temporal pole; Vermis 6,7,8 = cerebellum vermis lobule 6, 7 & 8 (Larsell's schema); Cereb 6 = cerebellum hemisphere lobule 6, left and right.



4.3.4 Regional volumetric difference in WM

The final effective smoothness for the smoothed modulated WM images was 8.5mm x 8.1mm x 8.0mm. Two bilateral and symmetric clusters were significantly greater in relative regional volume in the ASC compared to the NT group. Cluster 1 (k_e = 18627, peak voxel *T* = 5.78, MNI coordinate [34,-56,18]) spread across right temporal, parietal and occipital regions, and overlapped significantly with the passage of certain major association fibre tracts (i.e., arcuate fasciculus, cingulum, inferior longitudinal fasciculus), projection fibre tracts (i.e., internal capsule) and commissural fibre tracts (i.e., fibres extending from the posterior portion of the corpus callosum); cluster 2 (k_e = 17305, peak voxel *T* = 4.41, MNI coordinate [-16,-39,26]) involved a symmetric left cerebral hemisphere cluster that overlapped with the same tracts of the left side. See Figure 4-2 for details of the clusters overlaid on a standard space atlas of tractography-defined major WM tracts (Thiebaut de Schotten et al., 2011).

In the other direction, three clusters were significantly larger in the NT compared to the ASC group. Cluster 1 (k_e = 3867, peak voxel *T* = 3.92, MNI coordinate [-23,-40,-37]) located in the left part of pons and cerebellum, which overlapped significantly with the ponto-cerebellar portion of the right cortico-ponto-cerebellar tract; cluster 2 (k_e = 8033, peak voxel *T* = 3.86, MNI coordinate [18,-21,-29]) located nearly symmetrically to cluster 1 at the right side of pons and cerebellum; cluster 3 (k_e = 4270, peak level *T* = 3.44, MNI coordinate [30,-23,0]) involved the right side of parts of internal capsule at the level around basal ganglia, which was also part of the corticopontine portion of the right cortico-ponto-cerebellar tract. See Figure 4-3.

Figure 4-2 Larger relative regional WM volume in women with ASC

Female adults with ASC had greater relative regional WM volume in the clusters marked in light blue, compared to typical female adults. Significant clusters were overlaid on an averaged T_1-weighted template image; see Thiebaut de Schotten et al. (2011) for detail. The second to last columns (left to right) of panel A show the same clusters involving different major WM fibre tracts in axial (top row) and coronal (second row) views in neurological convention; numbers in green indicate MNI coordinates on the z-axis (for axial view) and y-axis (for coronal view). Panels B & C indicate the same clusters (as depicted in panel A) involving the left and right inferior longitudinal fasciculi, respectively; numbers in green indicate MNI coordinates on the x-axis. For the WM tract maps, regions in purple indicate significant probability of the tract existing in the voxel (FWE $p < 0.05$), regions in blue further indicate voxels showing the existence of the tract for which the percentage overlap amongst all 40 participants in the original study was greater than 50%, regions in yellow indicate percentage overlap > 75%, and regions in red indicate percentage overlap > 90%; see Thiebaut de Schotten et al. (2011) for detail.

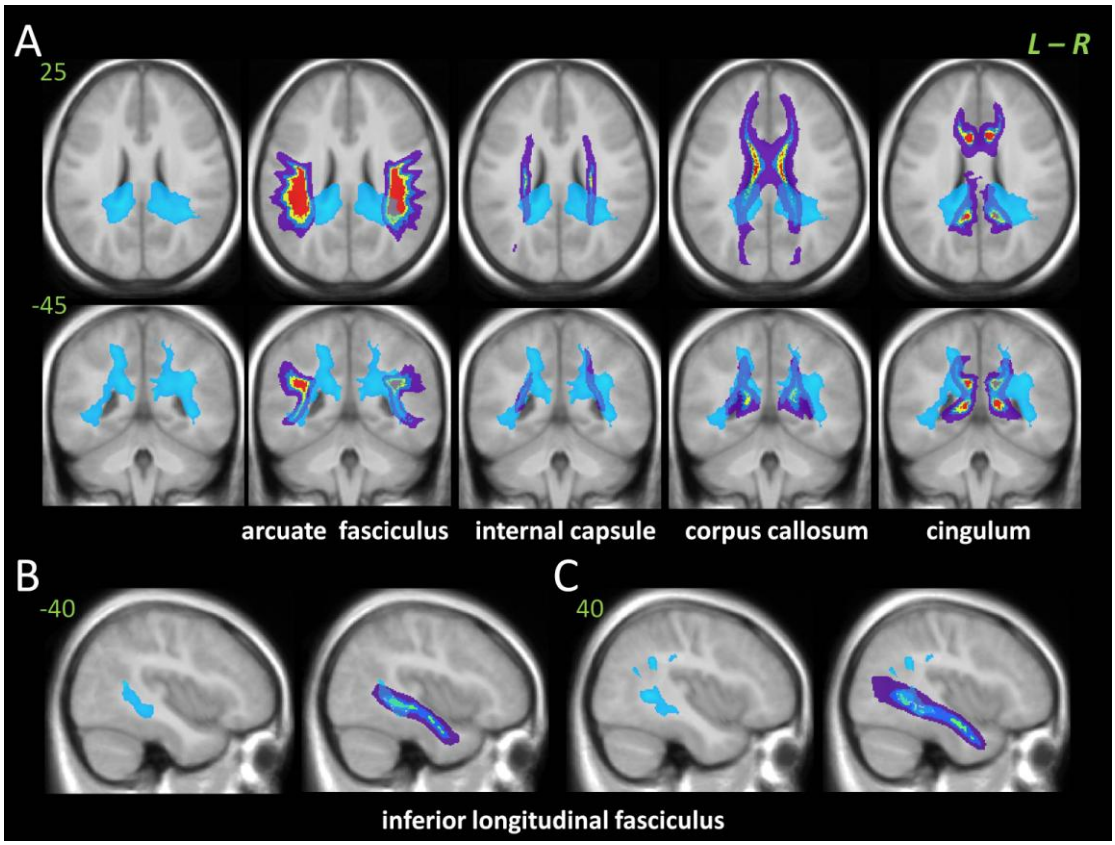
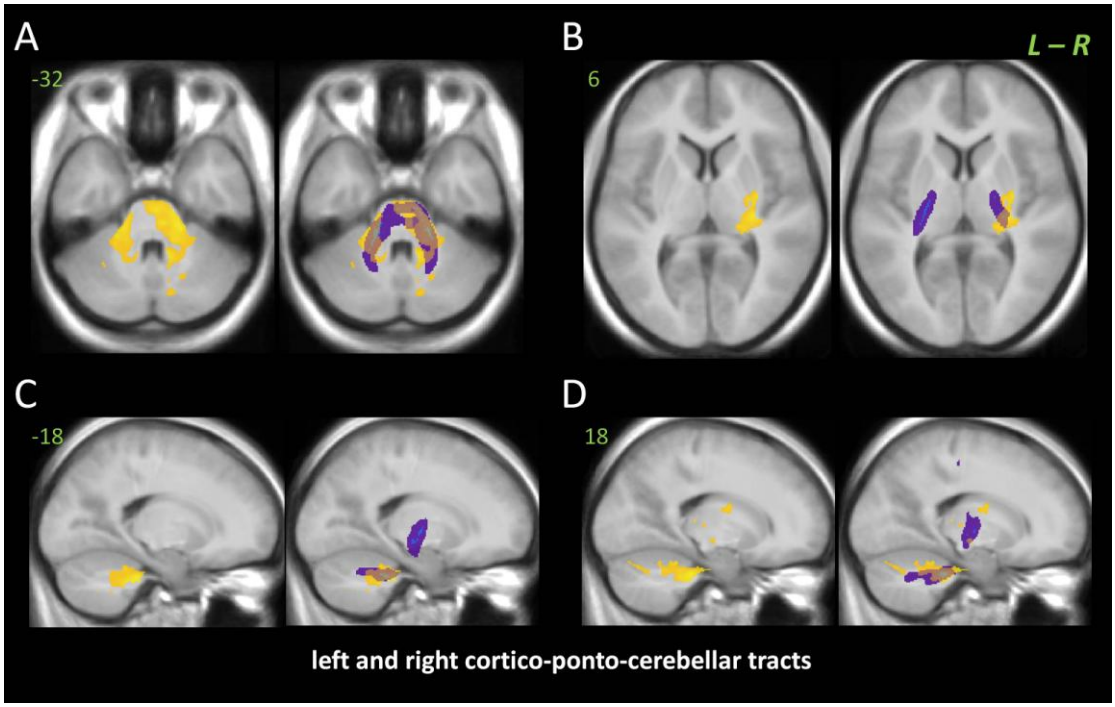


Figure 4-3 Smaller relative regional WM volume in women with ASC

Female adults with ASC had less relative regional WM volume in the clusters marked in orange, compared to typical female adults. The right slice for each panel indicates the same cluster as the left slice but overlaid on the cortico-ponto-cerebellar tracts. Panel A shows the axial slice at the level of pons and cerebellum and the involvement of the bilateral ponto-cerebellar fibres. Panel B shows the axial slice at the level around basal ganglia and the involvement of the right corticopontine fibres. Panels C & D, the left and right sagittal slices, recapitulate these involvements.



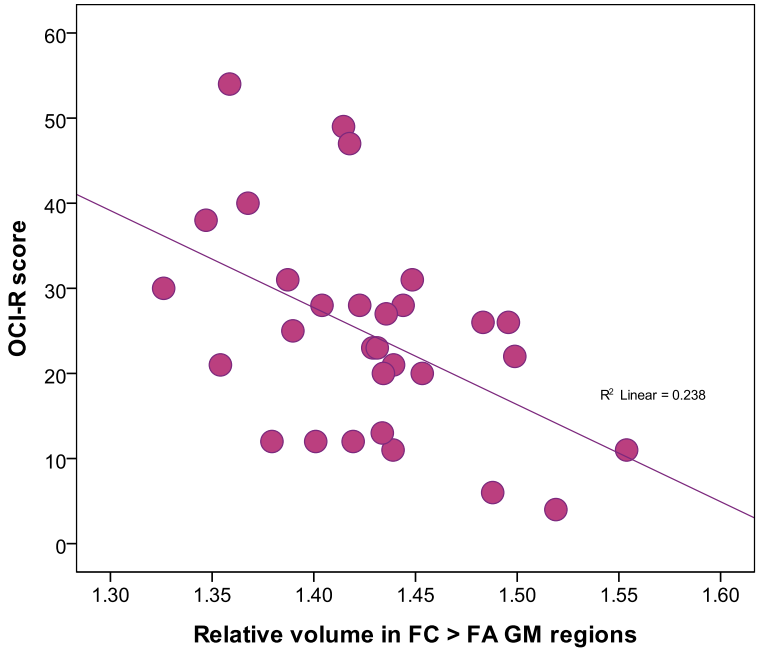
4.3.5 Correlation to behavioural measures

Within NT and ASC groups, respectively, the average relative regional volume of the GM and WM regions showing group differences did not correlate with any of the behavioural and cognitive measures outlined in Chapter 3, at a nominal level of $p < 0.05$. Particularly in the ASC group, there were no correlations between these volumes with measures of autistic symptoms or traits, including ADI-R and ADOS domain

sub-scores, AQ, EQ and SQ scores. The only exception was that the average relative GM volume of regions significantly smaller in ASC was negatively correlated with co-occurring obsessive-compulsive symptoms (OCI-R score) (r = -0.49, p = 0.006); see Figure 4-4. Due to multiple comparisons, this correlation was vulnerable to type I error and should be viewed as preliminary.

Figure 4-4 Correlation of relative GM volume (where ASC group was smaller) to OCI-R score, in the ASC group

Exploratory analysis showed that in the ASC group, the larger the relative volume of the structures significantly smaller than the NT group, the milder their co-occurring obsessive-compulsive symptoms. FC = neurotypical females; FA = females with ASC; OCI-R = Obsessive-Compulsive Inventory-Revised.



4.4 Discussion

In the largest sample to date, this study explored volumetric differences in local GM and WM between able female adults with and without ASC. Compared to neurotypical women, women with ASC had smaller relative (i.e., corrected for individual total) GM volume in bilateral ACC, MCC, SMA, insula, superior cerebellum, right temporal pole and calcarine. Regarding WM, women with ASC had larger posterior brain regions bilaterally involving a range of major tracts such as the arcuate fasciculus, internal capsule, corpus callosum, cingulum and inferior longitudinal fasciculus. However, they also had smaller relative WM volume in bilateral ponto-cerebellar fibres and the right corticopontine fibres. These morphometric differences provide a first definitive look at the neuroanatomical differences between female adults with and without ASC.

4.4.1 Comparison to previous imaging studies of females with ASC

Globally, the lack of a total GM or WM group difference is a replication of findings from a previous smaller study on adults (M. C. Craig, et al., 2007). However, toddler girls with ASC had larger GM volume than those without (Bloss & Courchesne, 2007). The lack of such a difference in adults meshes well with the idea that globally the autistic brain tends to be larger overall in early development, but as development progresses, their brains normalise allowing typically developing individuals to 'catch up' (Courchesne, et al., 2011; Courchesne, et al., 2007).

The localised group differences overlapped to some extent (especially for WM) with the prior study. This is noteworthy given that this study and Craig et al. (2007)

applied different MRI scanning protocols and field strengths (i.e., 3T, DESPOT1 vs. 1.5T, SPGR), preprocessing schemes (i.e., SPM8 segmentation and normalisation via DARTEL vs. SPM2 segmentation and normalisation), smoothing kernel size (i.e., 4mm vs. 5mm FWHM), statistical inference method (i.e., parametric statistics and topological FDR correction vs. non-parametric statistics with cluster-level false-positive threshold), covariates (i.e., with vs. without age as a covariate) and ways to assess relative regional volume (i.e., proportional scaling vs. including total volume as a covariate). Both reported decreased local GM volume in females with ASC compared to controls. Replicated regions included the right ACC, right superior temporal gyrus/temporal pole and the visual cortex (lingual and calcarine gyri). In terms of differences, instead of finding extensive involvements of lateral and medial temporal regions and the basal ganglia, we identified bilaterally the anterior and middle cingulate cortices (ACC/MCC), the supplementary motor area (SMA), insula and superior cerebellum. In terms of WM, both studies strikingly identified common regions in bilateral occipito-parieto-temporal regions that were larger in ASC than in controls, as well as a smaller pons in ASC. However, our study did not show other group differences identified by Craig et al. such as larger frontal and cerebellar clusters in ASC.

These partial inconsistencies could be due to reasons related to imaging methodology, but could also originate from the different age and IQ distributions of the samples. Both the ASC and the control participants in the Craig et al. study were on average 10 years older, and their full-scale IQ 10 points less than ours. This suggests that the samples across the two studies are representative of slightly different sub-populations of women with ASC in terms of their age and general cognitive

ability. However, our sample size is nearly twice the size of Craig et al., providing more statistical power to detect group differences. Another advantage of this study is the improved image registration algorithm (i.e., DARTEL) that minimises registration error across individuals. Despite these differences, what is significant, given the notoriously high inconsistency of VBM studies in males with ASC, is the replication of extensive WM regions showing the same pattern of group differences between females with and without ASC.

4.4.2 Relation to findings in males with ASC

In terms of GM, although the regions highlighted here have also been reported in previous imaging studies for male adults with autism (e.g. ACC and temporal pole), the directionality is opposite to the results in males (i.e., males with ASC show *larger* GM volume in fronto-temporal regions) (R. Chen, et al., 2011). More importantly, none of the regions identified in the Via et al. (predominantly male) meta-analysis overlap with the present GM findings. Similarly, for WM we hardly observed any overlap with regions summarised by Radua et al.'s meta-analysis.

It has been proposed that based on the idea that females are innately less vulnerable to developing ASC, more "severe" neuroanatomical changes or deviances are necessary for them to reach the point of needing to be clinically diagnosed (M. C. Craig, et al., 2007; Lord, et al., 1982; Murphy, et al., 2011; Wing, 1981). If this is true, what should be observed is that females with ASC show larger effect size of volumetric difference from typical females in the *same regions* as those found to be different between males with and without ASC, and/or females have broader spatial involvements over and above the regions revealed in the male ASC-control

comparison. The observed marked dissimilarity between adult males and females in terms of ASC-control differences in brain morphometry does not support this hypothesis. In fact this dissimilarity suggests a possible interaction between biological sex and ASC at the level of brain morphometry, illustrated in Chapter 1 as a *sex-specific effect* (SSE), indicating sex-specific neuroanatomical features for adults with ASC. A thorough formal investigation by contrasting matched four groups of males and females with and without ASC will be described in Chapter 5 to disentangle the relationship between sex and ASC in terms of brain anatomy.

4.4.3 Functional implications of the neuroanatomical features of females with ASC

Although we identified a range of smaller GM regions, as well as larger and smaller WM regions in ASC, no correlations were found between these relative regional volumes to autistic symptomatology or neuropsychological performances on a wide array of tasks (i.e., mentalizing, basic emotion recognition, response inhibition, visuospatial attention to detail and dexterity). The exception to this was a relationship with self-reported obsessive-compulsive symptoms that was nevertheless weak. Overall, this suggests that although women with ASC differ from typical women in terms of regionally specific brain structure, there is not a straightforward link between these structural abnormalities and variation at the cognitive and behavioural levels.

One explanation for the lack of structure-cognition/behaviour links is that the morphometric difference is simply a *neuroanatomical phenotype* reflecting diagnostic status. This phenotype could result from a variety of factors that are capable of shaping the growth of the brain, such as gene expression related to neuronal

patterning (Voineagu, et al., 2011), prenatal hormonal effects (Hines, 2005) or experiences of learning and training (Draganski et al., 2004; Driemeyer, Boyke, Gaser, Buchel, & May, 2008; Scholz, Klein, Behrens, & Johansen-Berg, 2009). Although one may expect functional correlates of this neuroanatomical phenotype, the multiple possible interacting factors and the long process of development across lifespan may have substantially complicated the picture. On the other hand, it could also be the case that our behavioural and cognitive measures were not sensitive or specific enough to capture the underlying functional correlation to these particular brain regions.

Although there was a lack of correlation with behavioural/cognitive measures, the brain regions showing a volumetric difference are known to carry out a wide array of specific functions. Discussion of these functions may allow for several directions of future work. In GM, ACC/MCC are vital to error detection and conflict monitoring (Botvinick, Nystrom, Fissell, Carter, & Cohen, 1999; Polli et al., 2005; S. F. Taylor et al., 2006), estimating probability and volatility (Behrens, Woolrich, Walton, & Rushworth, 2007) and evaluative decision making (Assadi, Yucel, & Pantelis, 2009; Bush et al., 2002). Social learning has also been shown to involve a parallel processing scheme at ACC/MCC gyrus (for social information) and at the cingulate sulcus, dorsal to ACC/MCC (for reward-based information) (Behrens, Hunt, & Rushworth, 2009; Behrens, Hunt, Woolrich, & Rushworth, 2008). The SMA is involved in motor planning and internal preparation (Grafton, Mazziotta, Woods, & Phelps, 1992), coordinating with ACC (Sahyoun, Floyer-Lea, Johansen-Berg, & Matthews, 2004). Subgenual ACC is a region rich in serotonergic neurons, coordinating intensely with autonomic systems responsible for drives control (hypothalamus and brain stem), limbic system regulating emotion (insula and

amygdala), hippocampus, prefrontal cortex, and having a pivotal role in the pathogenesis of depression (Insel, 2010). The insula is critical for bottom-up automatic affective (Adolphs, 2009; Ochsner et al., 2009; Wager et al., 2008) and evaluative processing (Cunningham et al., 2004), empathy (Singer et al., 2004; Wicker et al., 2003), interoceptive awareness (A. D. Craig, 2002; Critchley, Wiens, Rotshtein, Ohman, & Dolan, 2004) and attention (Uddin & Menon, 2009). Temporal pole is thought to be a semantic hub playing a role in memory, language, communication (Patterson, Nestor, & Rogers, 2007) as well as social cognition (C. D. Frith, 2007). Lastly, lingual and calcarine gyri are occipital structures responsible for visual information processing, and cerebellum involves extensively in motor coordination and executive function (D. L. Clark, Boutros, & Mendez, 2010).

On the other hand, it is much less straightforward to identify the functional correlates for WM structures revealed by structural MRI, due to the frequent multi-tract involvement and the lack of *a priori* knowledge from functional imaging studies. However by examining the involvement of tracts, it seems to echo the GM feature that tracts related to socio-emotional (i.e., cingulum, arcuate fasciculus and inferior longitudinal fasciculus) and visual (i.e., inferior longitudinal fasciculus and posterior corpus callosum) processing, as well as cortico-cerebellar coordination (i.e., internal capsule and cortico-ponto-cerebellar tracts) were partially involved. The exact nature of the WM group differences will benefit from further WM-specific investigation (e.g. diffusion imaging), which is one of our future research directions.

4.4.4 Larger versus smaller: better or worse?

The last question regards interpreting the direction of volumetric difference. For

GM, although a larger volume for typical adults (i.e., without neuropsychiatric conditions) is conventionally treated as reflecting an enhanced function or frequent use of the region that can be modulated by training (Fleming, Weil, Nagy, Dolan, & Rees, 2010; Kanai & Rees, 2011; Maguire et al., 2000), it is still unclear what a greater GM volume means in terms of its underlying microstructural and cellular basis (Kanai & Rees, 2011; Terrazas & McNaughton, 2000). There are several possible interpretations including higher number of neurons or glia cells, larger synaptic density, or more complex axonal or dendritic connections, etc. However there are also studies showing that GM volume is negatively correlated with cognitive performance in adolescents (Dumontheil, Hassan, Gilbert, & Blakemore, 2010), which could be explained developmentally as related to synaptic pruning (Kanai & Rees, 2011).

What underlies a volumetric change in ASC may be different from that of the typical population. An excess (or deficit) in volume may reflect something different about the development of people with ASC. For example, Courchesne and colleagues have proposed the idea of *early brain overgrowth plus possible later regression* (Courchesne, et al., 2011; Courchesne, et al., 2007), which suggests plausible age-specific neurobiological characteristics explaining morphological deviance from controls. Early brain overgrowth may reflect an excess in the number of neurons, while loss of neurons, neuroinflammation, degeneration and a whole host of other factors could explain reduction of size later in life (Amaral, Schumann, & Nordahl, 2008; Courchesne, et al., 2011). Hence for brains of adults with ASC, an excessive GM volume may reflect either (or a combination of) residual of early neuron excess, insufficient pruning or factors related to experience and training. A reduced GM volume may indicate regression, over-correction or underuse. What among these

possibilities contributes to the volumetric changes in ASC is still unknown. In addition, the hypothesis of *later regression* was proposed solely from data of male adults with ASC, and we should not assume the same will be true for female adults with ASC. One line of evidence supporting this idea that the same assumption for males should not be applied to females can be seen in early brain size, that girls with autism have even larger overgrowth than boys (Bloss & Courchesne, 2007; Schumann, et al., 2009; Schumann, et al., 2010). In sum, the interpretation of the direction of volumetric change in ASC, especially in females, remains open. It may relate to specific primary characteristics or developmental changes of ASC, or even influences from experience and learning. All these speculations, however, await future investigations applying different research strategies for clarification.

For cerebellum, which might show a different trajectory of growth pathology compared to the cerebrum (Courchesne, et al., 2011; Hallahan et al., 2009; Webb et al., 2009), our result actually corresponds well to previous findings of hypoplasia of vermis lobule 6 and 7 throughout the whole life span (Stanfield, et al., 2008). The functional implication is yet unclear.

WM has a different growth trajectory from late childhood to early adulthood, compared to GM (Giedd & Rapoport, 2010; Lenroot, et al., 2007). GM undergoes progressive volume reduction after its peak at early adolescence, which may reflect synaptic pruning or myelination along grey-white junction (Sowell, Thompson, Tessner, & Toga, 2001). WM volume instead increases continuously to adulthood, which may underlie enhanced connectivity. An excess in WM in adulthood may be viewed as reflecting enhanced growth due to experience and learning, which might be the case for female adults with ASC who often excel in “pretending to be normal”

(see Chapter 2 for discussion) (Attwood, 2007; Attwood & Grandin, 2006; Willey, 1999). Equally likely it may be the residual of the early brain overgrowth in WM. On the other hand, a deficit in WM volume (e.g. in cortico-ponto-cerebellar tracts) in ASC may indicate underdevelopment of the fibres.

Additionally, WM volume may be influenced by genetic and hormonal factors. Testosterone and androgen receptor activity affect growth of WM volume possibly on axonal calibre (Perrin et al., 2008), and affect brain maturation differentially for males and females in both GM (i.e., cortical thinning) (Raznahan, Lee, et al., 2010) and WM (Perrin, et al., 2008). Given WM growth trajectory is sexually dimorphic (i.e., males have steeper growth curves than females) (Giedd & Rapoport, 2010; Lenroot, et al., 2007), the regional increase of WM in female adults with ASC may reflect a region-specific "masculinisation" compared to typical women. This would be consistent with the extreme male brain (EMB) hypothesis. This in turn may reflect a possible role of sex hormones and related genetic factors in the presentation of females with ASC.

4.4.5 Limitations and future directions

There are several caveats and limitations to note about the current study. First, VBM is a mass-univariate statistical approach. Although unbiased and powerful in detecting volumetric differences, it is not able to delineate multivariate features of the brain such as shape. It has been shown that different geometric features of GM, such as cortical thickness and surface area, capture different biological processes in terms of their genetic determinants (Panizzon et al., 2009; Winkler et al., 2010), neurobiological underpinning (Huttenlocher, 1990; Rakic, 1988; Sowell et al., 2004),

developmental trajectory (Armstrong, Schleicher, Omran, Curtis, & Zilles, 1995; Sowell, et al., 2007) and evolutionary relevance (Rakic, 1995). Further investigation of the geometric features will provide fine-grained neuroanatomical characterisation of women with ASC. Second, our sample represents only a sub-population of women on the autism spectrum. Generalisation of the findings to other sub-populations (e.g. younger/older, with lower IQ or with comorbidities) may thus be limited. Third, although the sample is homogenous in terms of developmental maturity (all above 18), brain change still occurs in adulthood. In the analysis we only modelled chronological age as a nuisance covariate rather than specifically examining how it affects brain morphometry, and whether it has differential effects on the two groups (i.e., an interaction between age and diagnosis). This will be an interesting future direction to pursue, especially since differential chronological age effects have been observed in brain morphometry of males with and without ASC (Raznahan, Toro, et al., 2010).

Fourth, we did not match the two groups on the phase of their menstruation cycle and a significantly higher proportion of typical women were currently using oral contraceptives than women with ASC (NT: 15/30, ASC: 6/30, $\chi^2 = 5.934$, $p = 0.03$). Matching on this variable is difficult because this may result in a systematic bias in sample selection (i.e., women who are not sexually active might be more socially atypical). Furthermore, we know very little about how female sex hormones (e.g., estrogen, progesterone, luteinizing and follicle stimulating hormones) and their changes during the menstruation cycle affect brain structure. Although total GM volume may be influenced by the menstruation cycle (i.e., peaking at the time of ovulation) (Hagemann et al., 2011), there is no report on how local structures are affected. Further research should pay attention to how current hormonal status affects

brain structure, and whether this occurs differentially in females with and without ASC. Lastly, significantly more women with ASC were currently receiving medication for mood or anxiety disorders (NT: 3/30, ASC: 14/30, χ^2 = 9.932, p = 0.003), which was not surprising given their much higher symptom levels (see Chapter 3). Although we see this as reflecting the natural condition of ASC in women, it is an important future direction to illuminate how mood or anxiety disorders arise in ASC and how the brain changes correspondingly. Further discussion on co-occurring mood or anxiety disorders in ASC and sex differences in receiving medical treatments will be presented in Chapter 7.

Chapter 5

Disentangling How Sex Interacts with ASC:

Analysis on Brain Morphometry

5.1 Introduction

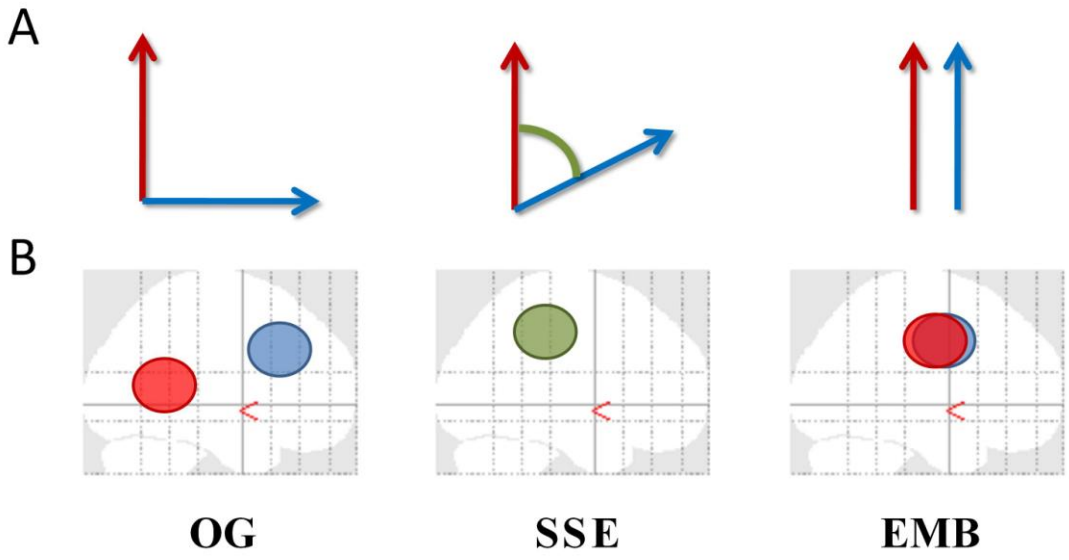
The previous chapter has shown that there are marked GM and WM volumetric differences between able female adults with and without ASC. On comparing these results with meta-analyses with predominantly male participants, a key observation is that differences in females do not overlap with differences consistently reported in males. This observation suggests that there may be sex-specific neuroanatomical phenotypes for ASC. Direct examination of this hypothesis requires formal comparisons of four groups (i.e., males with and without ASC, and females with and without ASC). This chapter will focus on this issue at the level of brain morphometry (VBM) from both empirical and theoretical perspectives.

5.1.1 Relationship between sex and ASC: Recapitulation of models

In Chapter 1 the competing and partially complementary models about the relationship between sex and ASC were introduced: orthogonal (OG), sex-specific effect (SSE) and the extreme male brain pattern (EMB). These models were applied to describe and test single cognitive/behavioural (univariate) measures in Chapter 3. To extend the models to the brain, not only single measures (e.g. a voxel) are tested, but they also have predictions for the *spatial characteristics* of the effects of sex and ASC. Figure 5-1 re-establishes the relationships. The following sections briefly recap how each model describes the univariate relationship, then explain what they predict in the brain.

Figure 5-1 Illustrating concepts of the relationships between sex and ASC

Panel A, a replication of Figure 1-3, illustrates the theoretical models for the relationships between sex (red arrow) and ASC (blue arrow) in a geometric sense. An OG model (left) indicates the two effects are orthogonal, i.e., independent from each other. An SSE model (middle) indicates the effect of one is dependent on the other, so there is an interaction between them (represented by the green curve indicating a non-orthogonal angle). An EMB model (right) indicates the two effects are in the same direction, i.e., showing the same effect; this actually merges the two dimensions (sex and ASC) into one. Panel B shows how these models, when applied to the brain, predict the effects spatially over the brain. The OG model predicts that regions showing effect of sex (red circle) and those showing effect of ASC (blue circle) are distinct. The SSE model predicts that there are regions showing sex-by-ASC interaction (green circle), and whether there are regions showing effects of sex and/or ASC does not matter. The EMB model predicts that the regions showing effect of sex and those showing effect of ASC overlap to a substantial extent.



5.1.1.1 Orthogonal (OG)

For a single variable (e.g. a cognitive measure or voxel value), if sex and ASC have independent effects, they are *orthogonal* (OG model, Figure 5-1 panel A, left). This means the effect of ASC on the variable does not depend on sex, and the effect of

sex on the variable is also not influenced by the diagnostic status.

For spatial characteristics in the brain, the OG model (Figure 5-1 panel B, left) has two predictions. First, in a two-way factorial analysis of variance (ANOVA) VBM analysis there will be no brain regions showing sex-by-ASC interactions (so the effects of sex and ASC are independent for each brain voxel). Second, the main effects of sex and ASC will be at spatially distinct regions (so sex and ASC affect different brain structures). Therefore the presence of sex-specific effects or extreme male brain patterns (see below) rejects this model.

5.1.1.2 Sex-specific effect (SSE): Interaction

For a single variable, if sex affects how ASC has an effect (or vice versa), then there should be an *interaction* between the two factors. In other words, how ASC affects the measure of interest (here the voxel value) is expressed differentially in males and females (i.e., dependent on sex). This can be referred to as a *sex-specific effect* (SSE model, Figure 5-1 panel A, middle).

For spatial characteristics, the model predicts the presence of regions showing significant sex-by-ASC interaction in a two-way factorial ANOVA VBM analysis (Figure 5-1 panel B, middle).

5.1.1.3 The extreme male brain (EMB) pattern: A special relationship derived from cognitive theories for autism

This concept arises from the *extreme male brain theory (EMB theory) of autism*, first suggested by Hans Asperger in his initial case reports (Asperger, 1944), followed by Lorna Wing (Wing, 1981) and formally proposed by Simon Baron-Cohen (Baron-Cohen, 2002; Baron-Cohen, Knickmeyer, et al., 2005). The theory suggests

that ASC is a hyper-masculinised presentation in terms of aspects of cognition that show sex differences in the general population. Nevertheless, how sex interacts with ASC is not described. Clarifying the central idea of the EMB theory, it suggests that for a measure of interest (i.e., cognitive abilities on empathy or systemizing), the effects of ASC and sex act in the same direction implied by the EMB theory (EMB model, see Figure 5-1 panel A, right). Therefore, the EMB model is different at the basic framework level from the SSE and OG models, that it is actually a *1-dimension* model, in contrast to the 2-dimension nature (i.e., sex and ASC) of the other two models.

Due to the fact that the EMB model is a 1-dimension model (i.e., sex and ASC converge as one dimension), it *cannot* be fully tested under the framework of a two-way factorial ANOVA for either a single variable (see Chapter 1 for detail) or spatial characteristics in the brain. For spatial characteristics, the only instance from a two-way factorial design VBM that confirms an EMB pattern is the presence of two main effects (sex and ASC) overlapping substantially on the same brain region and fulfils EMB-implied directionality (see Figure 5-1 panel B, right). However, this is not the only instance that matches potential predictions from the EMB theory. For example, one can imagine that the levels of factors such as sex and diagnosis line up in a linear fashion, without significant main effects but still showing an EMB pattern.

Since multiple predictions can be extended from the original EMB theory, which only provides a general idea that sex and ASC act along the same direction, the best way to test it is to follow strictly how an EMB pattern is defined at the univariate level. To illustrate this we first need to refer back to the cognitive EMB theory, which can be decomposed into three components:

1. There is a typical sexual dimorphism (e.g., typical males perform worse than typical females on empathy tasks).
2. Males with ASC (MA) are more *masculinised* compared to typical male controls (MC) (e.g., MA perform even worse than MC on empathy tasks).
3. Females with ASC (FA), though not explicitly described in the original theory, should perform similarly to MA thus are also more *masculinised* compared to typical female controls (FC) (e.g., FA perform worse than FC on empathy tasks).

It is important to note that the term "*masculinised*/*masculinisation*" used throughout this chapter is simply descriptive of a pattern defined as the differences between typical males (MC) and typical females (FC), including both directions (i.e., MC > FC and MC < FC).

Following these requisites, the spatial characteristics predicted by the EMB model will be shown by *spatial overlap analyses on planned pair-wise*[6] *comparisons*. For single measures, only the above-mentioned relationships regarding the matching on *directionality (of differences)* need to be attained. To meet the EMB model on the brain level the *spatial distribution* of these differences is equally crucial, and the pair-wise planned comparisons need to show:

1. There is a typical sexual dimorphism in the brain (e.g., for regional volumetric difference measured by VBM, MC > FC in region X).
2. The map of differences between MA and MC has *the same directionality* (i.e.,

[6] As mentioned earlier in Chapter 3, the term *pair-wise* used throughout this dissertation indicates a planned comparison between two *independent groups*, but *not* "paired (dependent) groups". The statistical tests performed all treated the two groups as independent.

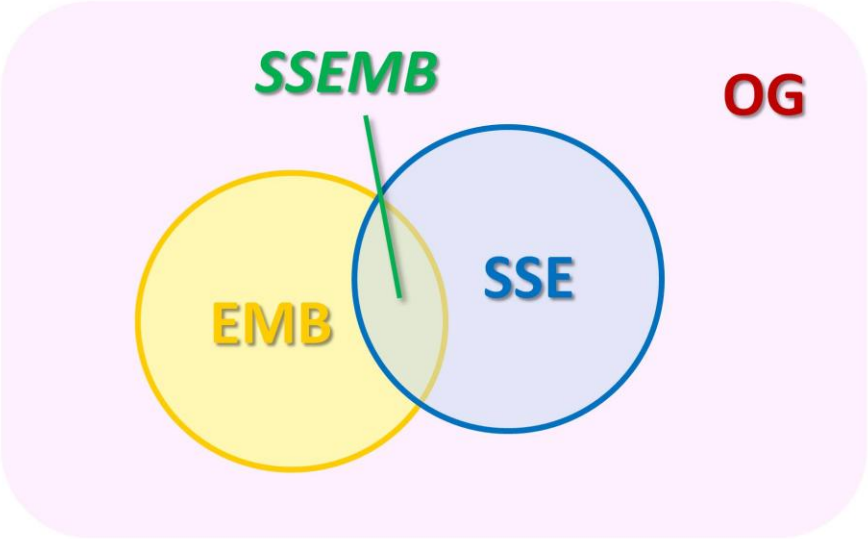
masculinised) as and *overlaps in spatial distribution* with the map of differences between MC and FC (e.g., MA > MC in region Y, and Y overlaps with X).

3. The map of differences between FA and FC has *the same directionality* (i.e., masculinised) as and *overlaps in spatial distribution* with the map of differences between MC and FC (e.g., FA > FC in region Z, and Z overlaps with X).

Point 1 is the prerequisite for the tests on EMB pattern to be viable. If point 1 and both point 2 and 3 are fulfilled then an EMB pattern across sexes (i.e., a *full-EMB pattern*) will be supported. This full-EMB pattern will likely be captured by a two-way factorial design VBM as overlap of the two main effect maps (Figure 5-1 panel B, right). However, if point 1 and only one of point 2 or 3 is fulfilled, then it indicates a *partial* EMB pattern that presents only in one sex. This is conceptualised as a *sex-specific EMB pattern* (SSEMB model, see Figure 5-2 for explanation and contrasts to EMB, SSE and OG models).

Figure 5-2 Further illustration on the statistical relationships between sex and ASC

If sex and ASC are independently affecting an outcome measure they are orthogonal (OG). Using a two-way ANOVA we can test if there is a statistical interaction between them (SSE); the presence of SSE will reject the OG model. If the effect of ASC actually acts as the effect of sex (i.e., masculinisation) then they show a relationship of EMB, which cannot be adequately tested by the ANOVA framework. Planned pair-wise comparisons can instead examine if the effect of ASC acts as the effect of masculinisation in males and females, respectively. If this happens the same way in both sexes it is full-EMB; if this happens only in one sex but not in the other it is sex-specific, hence SSEMB. Specifically, if this sex difference (in whether there is an EMB pattern) is large enough, it will be captured statistically as an SSE in the two-way ANOVA. Note that SSEMB is just one possibility amongst many SSEs. At the brain level, full-EMB will present itself in the two-way ANOVA VBM as a significant spatial overlap of the two main effect maps. EMB = "extreme male brain" model; OG = "orthogonal" model; SSE = "sex-specific effect" model; SSEMB = "sex-specific extreme male brain" model.



5.1.2 Research questions and tests at the brain level

This chapter will compare the neuroanatomy in terms of VBM on four age- and IQ-matched groups of male and female adults with and without ASC, to clarify the relationships between sex and ASC. Both *two-way factorial design VBM analysis* and *spatial overlap analyses on planned pair-wise VBM comparisons* were performed to test which model(s) best describes the spatial characteristics. Local brain regions particularly showing the effects of the best model(s) will also be identified and discussed.

5.2 Methods

5.2.1 Participants

Thirty female adults with ASC and 30 typical female adults were included, who were the same participants analysed in Chapter 4. The male participants were recruited via the MRC AIMS project by the same inclusion and exclusion criteria as for the females, and were scanned during the period of July 2007 to November 2008, using exactly the same MRI machine, head coil, version of operating system, imaging protocol and pulse sequences as the protocol for the female participants described in Chapter 4. Forty-five male adults with ASC took part and 33 were included for analysis since they scored above the ADI-R cut-offs for autism by the same criteria described in Chapter 2. Two participants were excluded (one with an incidental finding of agenesis of corpus callosum, and one due to motion artefact resulting in poor image quality), leaving 31 available participants. For the control group, 33 typical male adults participated during the same period. Among them two were

excluded due to motion artefact, leaving 31 available participants. Finally, 30 were selected from each male group to match for age and IQ to the two female groups. The final sample comprised of 120 participants in four age- and IQ-matched equal-sized groups (N=30 each).

5.2.2 Behavioural assessments

All participants were assessed using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) which measures verbal, performance and full-scale IQ, along with a series of self-report and neuropsychological assessments (details given in Chapters 2 and 3). All ASC participants were assessed by the ADI-R and ADOS.

5.2.3 Digit ratio measurement

Given the interest in hormone-related factors which might contribute to possible masculinisation, for all female participants we measured their second and fourth finger lengths on both hands, in order to calculate the second-to-fourth digit ratio (2D:4D ratio), held to be a proxy of prenatal androgen exposure effect (Breedlove, 2010; Manning, Baron-Cohen, Wheelwright, & Sanders, 2001; Manning, Scutt, Wilson, & Lewis-Jones, 1998) which included the influences from both circulating androgen level (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004) and the activity of androgen receptor genes (Manning, Bundred, & Flanagan, 2002; Manning, Bundred, Newton, & Flanagan, 2003). Finger length can be measured directly or from photocopies, both with high reliability (Robinson & Manning, 2000). However measures from photocopies are significantly smaller than direct measures

(Manning, Fink, Neave, & Caswell, 2005) hence the two methods cannot be combined within the same study. Here we adopted the photocopy method, which provided the opportunity to check for reliability on measurements. Participants' hands were photocopied using the same high-resolution photocopier. While copying they were asked to have their hands wide open and pressed flat on the glass of the machine. Photocopies were examined to ensure satisfactory quality for accurate identification of the creases and finger tips, and repeated if not satisfactory. Finger lengths of the second and fourth digits of both hands were measured from the midpoint of the basal crease to the tip of the finger with the same electronic vernier calliper reading to 0.01 mm, which has been shown to be a very reliable measure (Manning, et al., 1998).

One trained researcher measured all the hands from photocopies twice. An intra-rater reliability analysis on 105 participants, including the 60 female participants here, was conducted and showed excellent agreement for measurements for all the four digits (intra-class coefficient > 0.99). The average of the two repeated measurements of each finger was used as the final measure of finger length to calculate the 2D:4D ratio for the left and right hands, respectively.

5.2.4 Structural MRI data acquisition and processing

Details of the scanning protocol and processing are given in Chapter 4. The only difference was on registration and normalisation of the segmented GM and WM images to the study-specific template by SPM8 and DARTEL (Ashburner, 2007), we used all 120 participant images to generate the study-specific template. This was required by DARTEL processing and ensured that all four groups were registered the same way and could be compared directly without bias.

5.2.5 Two-way factorial design VBM analysis

In this omnibus VBM analysis all four groups were compared under a two-way factorial framework, separately for GM and WM. In brief, a general linear model (ANCOVA) was fitted at each voxel where sex and diagnosis were the fixed factors, and age (linear term) was the nuisance covariate to remove variance in relative local brain volume explained by age. Statistical outcomes were corrected for multiple comparisons by controlling topological false discovery rate (FDR) calculated under the Gaussian Random Field Theory (Chumbley & Friston, 2009). Here we used a cluster-forming height threshold of $p < 0.025$ for each contrast and a spatial extent threshold that ensures a FDR at $q < 0.05$ for clusters. All the above analyses were conducted using the SPM8 software.

5.2.6 Spatial overlap analyses on planned pair-wise VBM comparisons

Each planned VBM comparison was carried out with exactly the same statistical strategy and threshold as in Chapter 4. Three sets of VBM comparison (MC – FC, MA – MC, FA – FC) on relative GM and WM volumes were performed, with two contrasts for each comparison (e.g. for MC – FC, there were MC > FC and FC > MC).

For measuring spatial overlap, we took the group difference maps applied with only height threshold ($p < 0.025$, uncorrected) but without spatial extent thresholding. This is because using a topological FDR procedure to control for type I error will result in different spatial extent thresholds for different VBM comparisons, potentially influencing further overlap analyses across group difference maps. We did not instead arbitrarily apply a same extent threshold (e.g. 100 voxels) to all the group difference

maps owing to the nature of this work in examining spatial overlap, that we would like to observe how the overlapping voxels were spatially distributed (i.e., contiguous or dispersed). The selection of the height threshold ($p < 0.025$, uncorrected, as in Chapter 4), though arbitrary and relatively lenient, helps avoid the type II error which is critical for this analysis given possible low effect size of group differences between the male groups, as implicated by the inconsistent results from previous VBM studies. Due to the multiple pair-wise VBM performed, there would be likely more false-positive voxels. Nevertheless the goal for this particular analysis was detecting spatial overlap of two maps. The statistical procedure to determine how likely the overlap occurred by chance would ensure that the overlap, if significant, was not due to false-positive voxels in the two maps (see below).

We investigated four sets of spatial overlap (see Figure 5-3) for GM and WM, respectively. For each overlap analysis, voxel number of each group difference map was first counted, then intersection voxels of the two group difference maps identified and numbers counted. Dividing the number of intersection voxels by voxel number of each group difference map gave two percentages (i.e., the percentage of intersection voxels occupying the first map, and the same for the second map). The two percentages together, or their average, indicate the extent of overlap of the two maps.

We then tested the probability that the overlap occurred by chance by two procedures. First, to test *how likely the overlap was generated from false-positive voxels in each observed group difference map*, a null distribution was built by calculating the overlap of two images of randomly generated *t*-values (from Student's *t* distribution) and thresholded at the same thresholds as the two observed maps, for 5,000 times. A *p*-value (P_{FPV}) was derived by finding the proportion of percentage

overlap in this null distribution higher than the observed value. Second, to test *how likely the observed overlap occurred by chance from two maps with fixed numbers of suprathreshold voxels as the observed group difference maps*, another null distribution was built by calculating the overlap of two images thresholded as the observed group difference maps but their brain voxels spatially randomly permuted, for 5,000 times. A second *p*-value (P_{RanOvp}) was derived by finding the proportion of percentage overlap in the null distribution higher than the observed value. All the measurements and statistical analyses were carried out with MATLAB version 2007b (The MathWorks Inc., Natick, MA, USA).

Pearson's correlation was used to explore the correlation between relative volume of the intersecting regions to digit ratio. These statistical analyses were performed with the PASW Statistics version 18 (SPSS Inc., Chicago, IL, USA).

Figure 5-3 Strategy of spatial overlap analyses on planned pair-wise VBM comparisons to test the EMB pattern in the brain

To test if an EMB pattern exists in male and female groups, respectively, we performed spatial overlap analyses on the group difference maps between the contrasts on the directionality implicated by the EMB model. MC – FC refers to the VBM comparison between typical male and female groups, which provides the spatial distribution map of ***masculinisation****. MA – MC refers to the VBM comparison between males with and without ASC. FA – FC refers to the VBM comparison between females with and without ASC. For each set of comparison (comparisons M and F, respectively), spatial overlap was counted only for the two combinations of contrasts that were on EMB-implicated directionality (e.g., MA > MC and MC > FC). The two "% overlap" for each set of comparison jointly provide information estimating the extent that an EMB pattern exists. The associated p values (see above text) indicate the probability that this overlap occurs by chance. A summary estimate of overlap for each set of comparison can then be defined as the average of the percentages. The higher percentage overlap, the more similar spatial distribution of the two group difference maps is.*

VBM group difference maps	MA – MC	*Comparison M: EMB in males*	MC – FC
Contrasts	MA > MC	*% overlap (p)*	MC > FC
	MC > MA	*% overlap (p)*	FC > MC

VBM group difference maps	FA – FC	*Comparison F: EMB in females*	MC – FC
Contrasts	FA > FC	*% overlap (p)*	MC > FC
	FC > FA	*% overlap (p)*	FC > MC

5.2.7 Validation analysis for the male groups

For a validation analysis (see results section for reasons and details) specifically using the male ASC-control group difference maps generated from a much larger sample (to gain higher power to detect group differences in males), images from a multi-centre sample of the MRC AIMS project (Ecker, et al., in press) composed of 84 typical male adults (London: 38, Cambridge: 31, Oxford: 15) and 84 male adults with ASC (London: 38, Cambridge: 29, Oxford: 17) matched on age (NT: 28.0, SD = 6.3;

ASC: 26.1, SD = 7.1; t = 1.825, p = 0.070) and full-scale IQ (NT: 114.2, SD = 12.5; ASC: 110.5, SD = 14.2; t = 1.784, p = 0.076) were compared by VBM. Twenty-eight ASC and 29 typical control males in our cohort were included in this larger sample; slight discordance of participants was due to group matching reasons. All the image preprocessing steps, statistical methods and thresholds were the same as mentioned above except (i) the registration and normalisation by DARTEL took only these 168 male participants, (ii) in the general linear model for VBM, besides age, centre (i.e., scanning machine) was also included as a covariate.

5.3 Results

5.3.1 Behavioural characteristics

The four groups were matched on age and full-scale IQ; for subscales they were matched on verbal IQ, but MC scored higher than FA on performance IQ under a non-corrected threshold of $p < 0.05$. For self-report ASC traits, the pattern of group difference was the same as reported in Chapter 3 (on a slightly larger sample, N=32 per group). MA scored slightly higher than FA on the ADI-R communication and RSB domain scores under a non-corrected threshold of $p < 0.05$, but they had the same level of reciprocal social interaction score. As reported in Chapter 2, MA scored significantly higher than FA on the ADOS even after correction for multiple comparisons. See Table 5-1.

Table 5-1 Behavioural characteristics for the four groups

N=30 per group	MC Mean (SD)	MA Mean (SD) [range] [$]	FC Mean (SD)	FA Mean (SD) [range] [$]	Statistics [&]
Age (Years)	28.2 (5.6)	27.2 (7.3)	27.5 (6.5)	27.8 (7.6)	ns
Verbal IQ	112.7 (9.7)	114.3 (12.9)	118.5 (9.6)	115.8 (13.1)	ns
Performance IQ *	118.5 (11.6)	113.3 (15.0)	117.0 (9.3)	110.4 (16.7)	MC>FA (p = .021)
Full IQ *	117.5 (10.7)	115.4 (14.1)	120.2 (8.0)	114.9 (13.8)	ns
AQ	15.6 (6.9)	32.7 (7.3)	12.0 (4.8)	37.5 (6.7)	◎
EQ	42.7 (11.9)	19.7 (10.1)	53.5 (9.5)	19.5 (7.5)	◎
SQ *	60.3 (20.7)	68.3 (23.7)	47.0 (18.3)	73.5 (29.3)	◎
ADI-R [#]					
Social	–	18.0 (5.1) [10 – 27]	–	16.4 (4.3) [11 – 26]	ns
Communication	–	15.3 (3.5) [8 – 22]	–	13.1 (3.9) [8 – 22]	MA>FA (p = .029)
RSB *	–	5.6 (2.5) [2 – 10]	–	4.3 (1.7) [2 – 8]	MA>FA (p = .023)
ADOS [@]					
S + C	–	8.5 (5.0) [1 – 17]	–	4.3 (3.6) [0 – 13]	MA>FA (p < .001)
RSB	–	1.0 (1.0) [0 – 4]	–	0.1 (0.3) [0 – 1]	MA>FA (p < .001)

[$]: For ADI-R and ADOS scores.

[&]: Independent sample *t*-tests. All *p* values were **not** corrected for multiple comparisons.

*: Levene's Test for Equality of Variances showed significant non-equal variances, therefore equal variance was not assumed in the statistical tests.

[#]: N = 30 for MA, N = 28 for FA.

[@]: Distribution of scores significantly deviant from normal, therefore non-parametric Mann-Whitney tests were performed for group comparison of ADOS algorithm scores.

MC = (neuro)typical control group male adults; MA = male adults with ASC; FC = (neuro)typical control group female adults; FA = female adults with ASC; SD = standard deviation; ns = non-significant ($p > 0.05$); ◎ = the same statistical pattern among groups as described in Chapter 3, see the result section of Chapter 3 for detail; AQ = Autism Spectrum Quotient; EQ = Empathy Quotient; SQ = revised Systemizing Quotient; ADI-R = Autism Diagnostic Interview-Revised; RSB: repetitive, restrictive and stereotyped behaviour; ADOS: Autism Diagnostic Observation Schedule; S + C: ADOS

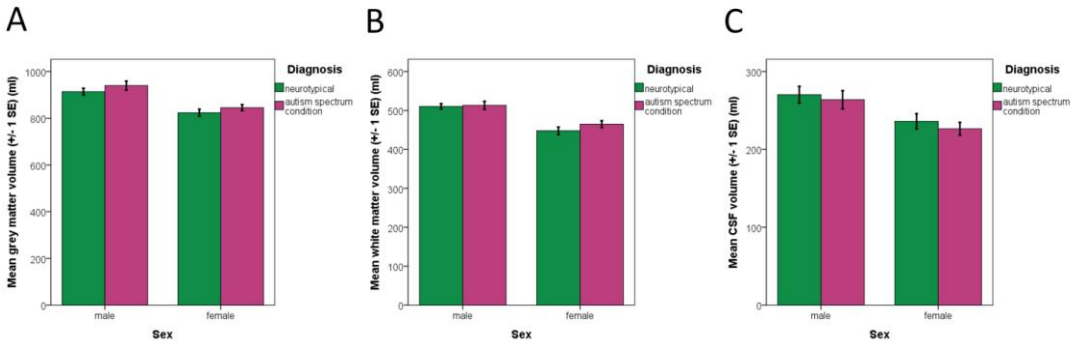
"social interaction + communication" total scores.

5.3.2 Global brain volume

A multivariate analysis of variance (MANOVA) treated total GM, WM and CSF volumes as dependent variables, and sex (two levels: male and female) and diagnosis (two levels: NT and ASC) as fixed factors. There was a significant main effect of sex (Pillai's Trace $V = 0.289$, $F_{(3,114)} = 15.478$, $p < 0.001$) but not of diagnosis or their interaction. Post-hoc univariate two-way factorial ANOVAs showed that this main effect of sex (i.e., Male > Female) was evident universally for GM ($F_{(1,116)} = 35.623$, $p < 0.001$, $\eta_p^2 = 0.235$), WM ($F_{(1,116)} = 38.727$, $p < 0.001$, $\eta_p^2 = 0.250$) and CSF ($F_{(1,116)} = 12.464$, $p = 0.001$, $\eta_p^2 = 0.097$) volumes, after Bonferroni correction for multiple comparisons. See Figure 5-4 for illustration.

Figure 5-4 Total GM, WM and CSF volume across the four groups

For GM (panel A), WM (panel B) and CSF (panel C), they all showed the same group difference pattern that males (left bars in each panel) had larger volume than females (right bars), in both NT (green) and ASC (purple) groups. Error bars indicate standard error of the mean.



Participants' chronological age was significantly negatively correlated with total GM (but not WM or CSF) volume in MC (r = -0.53, p = 0.002) and MA (r = -0.64, p < 0.001) groups. However, in FC age did not correlate with total GM volume but marginally positively correlated with WM (r = 0.34, p = 0.070) and CSF (r = 0.37, p = 0.043) volumes; in FA age was only marginally negatively correlated with total GM (r = -0.31, p = 0.101), and marginally positively to total WM (r = 0.27, p = 0.146) and CSF (r = 0.31, p = 0.093) volumes.

5.3.3 Two-way factorial design VBM

5.3.3.1 Grey matter

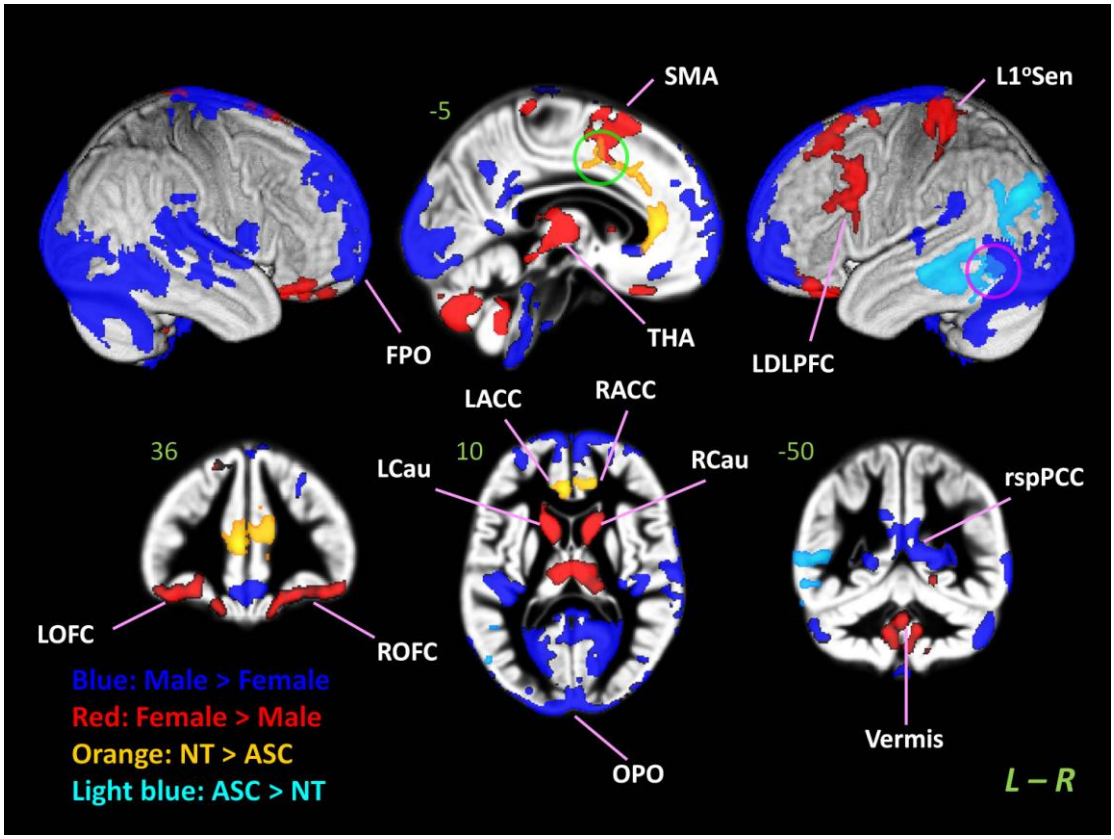
For GM, the final effective smoothness for the 4mm FWHM kernel-smoothed modulated GM images was 7.9mm x 7.6mm x 7.8mm, which was sufficient for statistical inferences based on the Gaussian Random Field Theory to be valid given the original voxel size (1mm isotropic). The two-way factorial design VBM showed significant main effects of both sex and diagnosis in spatially distributed regions, however no regions with significant interaction survived topological FDR correction; see Figure 5-5. Males had larger relative GM volume than females in eight clusters distributed at the bilateral frontal and occipital poles, occipito-temporal and temporo-parietal regions, ventromedial prefrontal cortex, subgenual anterior cingulate cortex, posterior cingulate cortex, cerebellar hemispheres and brain stem. Females had larger relative GM volume than males in nine clusters involving left dorsolateral prefrontal cortex, supplementary motor area, primary sensory cortex, and bilateral orbitofrontal cortices, caudate, thalamus, fusiform, hippocampal and parahippocampal gyri, cerebellar vermis and hemispheres (inferior lobules). Typical controls (NT) were

larger than the ASC group at one cluster involving bilateral anterior cingulate cortices and supplementary motor areas. ASC were larger than the NT group at two clusters involving left temporo-parieto-occipital regions.

The main effect maps of sex and ASC were for the most part spatially distinct from each other. However, there were still overlapping regions in SMA (Figure 5-5, green circle, overlap of Female > Male and NT > ASC maps) and temporo-occipital junction (purple circle, overlap of Male > Female and ASC > NT maps). Furthermore, they followed EMB-implicated directionality.

Figure 5-5 Main effects of sex and ASC in GM

Two-way factorial design VBM in GM demonstrated spatially distinct main effect maps of sex and ASC, however they overlapped in limited areas following EMB-implicated directionality (green circle: a region in SMA, overlap of Female > Male and NT > ASC maps; purple circle: a region in temporo-occipital junction, overlap of Male > Female and ASC > NT maps). Maps were overlaid on the GM segment of the study-specific template image generated by DARTEL. No regions with interaction effect survived multiple comparison correction. FPO = frontal pole; L1°Sen = left primary sensory cortex; LACC = left anterior cingulate cortex; LCau = left caudate; LDLPFC = left dorsal lateral prefrontal cortex; LOFC = left orbitofrontal cortex; OPO = occipital pole; RACC = right anterior cingulate cortex; RCau = right caudate; ROFC = right orbitofrontal cortex; rspPCC = retrosplenial posterior cingulate cortex; SMA = supplementary motor area; THA = thalamus; Vermis = cerebellar vermis.



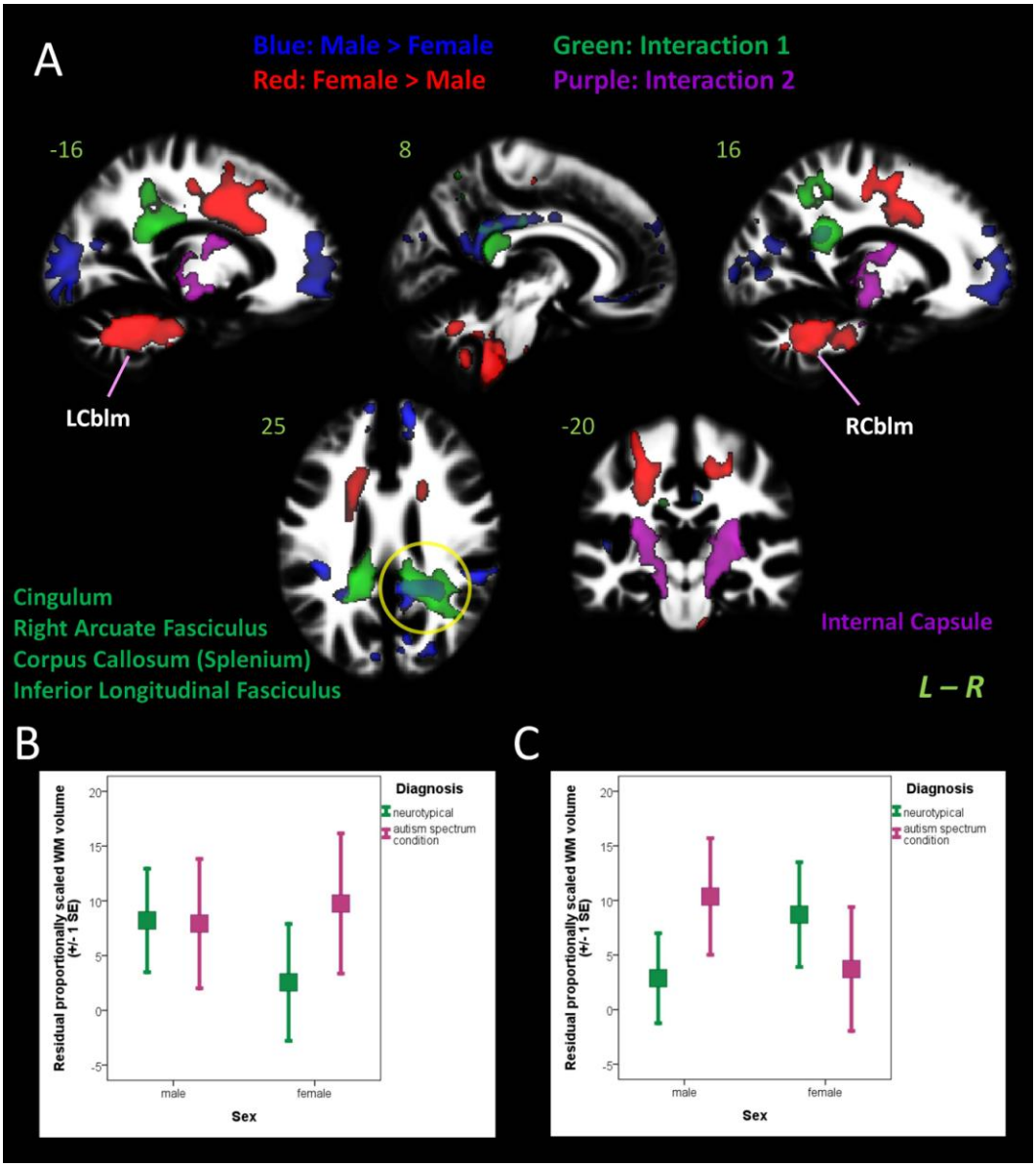
5.3.3.2 White matter

For WM, the final effective smoothness was 8.6mm x 8.1mm x 8.7mm. The two-way factorial design VBM revealed significant main effect of sex but not of diagnosis; see Figure 5-6. Males had larger relative WM volume than females in six clusters bilaterally distributed at the frontal, occipital and occipito-parieto-temporal junction regions in each hemisphere. Females had larger relative WM volume than males in three clusters, including one at the cerebellum and brainstem, and two bilaterally at the posterior part of frontal lobe involving fibres from the body of corpus callosum and the projection fibres of internal capsule. Furthermore, there were regions showing significant sex-by-ASC interaction (see below) which overlapped significantly with the Male > Female main effect map in the occipito-parieto-temporal junction region (especially at the right side, see yellow circle in Figure 5-6).

Both directions of significant interaction were noted. A pattern of FA>FC but MA=MC (Figure 5-6, panel B) was identified at bilateral occipito-parieto-temporal junction regions, which involved the posterior portion of bilateral cingulum, inferior longitudinal fasciculus (ILF), corpus callosum (splenium) and right arcuate fasciculus (AF). Another pattern of MA>MC but FA<FC (Figure 5-6, panel C) was identified at bilateral internal capsule, at the level around basal ganglia and thalamus. Methods to identify the involvement of WM tracts were described in Chapter 4, using a standard space atlas of tractography-defined major WM tracts (Thiebaut de Schotten, et al., 2011)

Figure 5-6 Main effects of sex and ASC and their interaction effects in WM

Panel A shows that the two-way factorial design VBM in WM demonstrated only a main effect of sex, but no main effect of diagnosis survived topological FDR correction. There were also regions showing significant interaction effects. Maps were overlaid on the WM segment of the study-specific template image generated by DARTEL. Regions in one direction of interaction involved posterior aspects of several tracts (in green, names given at the left lower corner of panel A), for which the pattern of group difference is illustrated in panel B. Regions in the other direction of interaction involved bilateral internal capsule (in purple, right lower corner of panel A), for which the pattern of group difference is illustrated in panel C. The yellow circle indicates a spatial overlap of the main effect of sex (Male > Female) and an interaction effect. LCblm = left cerebellum; RCblm = right cerebellum.



5.3.4 Spatial overlap in planned pair-wise VBM comparisons

5.3.4.1 The "masculinisation pattern"

The comparison between MC and FC groups provided the maps of typical sexual dimorphism (i.e., the "*masculinisation* pattern") that is the prerequisite for identifying if an EMB pattern exists in the brain. The observed typical sex difference (Figures 5-7 to 5-10, right columns of panels A and B) largely included regions reported by previous studies on sexual dimorphism (see Chapter 1 for detail) (Goldstein, et al., 2001; Good, et al., 2001; Luders, et al., 2009; Luders & Toga, 2010). In terms of GM, males were larger at the brain stem, lateral part of superior cerebellum, occipital pole (extending to right occipito-temporal junction), posterior cingulate, part of frontal pole and temporal poles; females were larger at the cerebellar vermis, thalamus, ACC, SMA, OFC, left hippocampal and parahippocampal gyri and superior frontal gyrus. For WM, males were larger at the anterior frontal and occipito-parieto-temporal junction regions; females were larger at the posterior frontal regions, ponto-cerebellar fibres and medulla oblongata. Note that without spatial extent thresholding the maps have 'salt-and-pepper' patterns of tiny clusters of voxels spreading out across space that are likely to be false-positives. However, these maps were used in the spatial overlap analyses for reasons described earlier in the methods section.

5.3.4.2 Spatial overlap of masculinisation and ASC effect in males and in females: Grey matter

Regarding the question "*Do males with ASC have masculinised brains?*" the intersection of MA – MC (i.e., *ASC effect* in males only) and MC – FC (i.e., *masculinisation*) group difference maps resulted in little spatial overlap (0.13% in

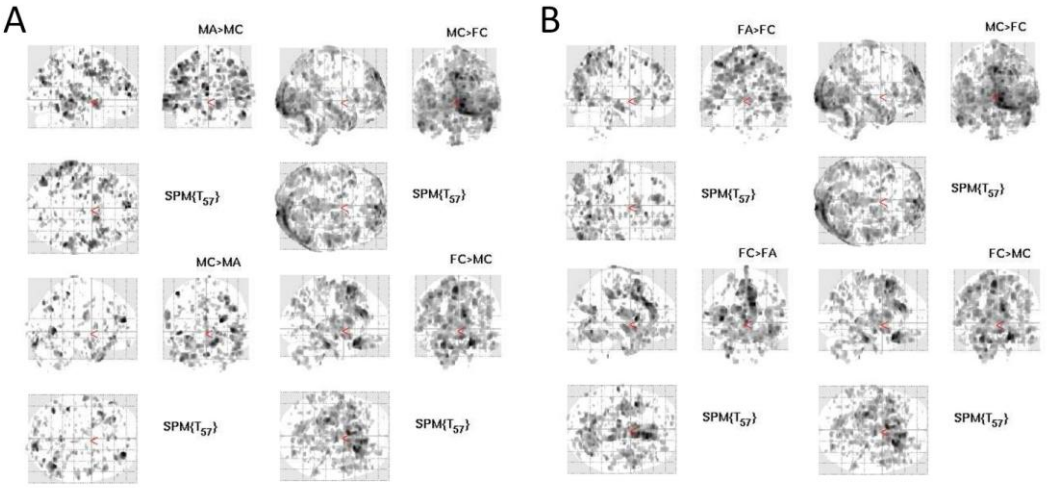
average; see Figure 5-7, panels A and C). This observed value of percentage overlap was smaller than any value in the null distribution of overlaps of pure false-positive-voxel maps (P_{FPV} = 1), suggesting negligible intersection generated from two non-noise group difference maps. Furthermore, the observed percentage overlap was smaller than any value in the null distribution of overlaps of permuted same-suprathreshold-voxel-number maps (P_{RanOvp} = 1). This indicated that the observed intersection was not only *not occurred by chance*, but was in fact a *significant non-overlap*.

In sharp contrast, concerning the question "*Do females with ASC have masculinised brains?*", the FA – FC (i.e., *ASC effect* in females only) and MC – FC group difference maps spatially overlapped to a substantial extent by an average of 20% (see Figure 5-7, panels B and C). This observed percentage overlap was *larger* than any value in the null distribution of overlaps of pure false-positive-voxel maps (P_{FPV} < .0002), suggesting that it was *not generated by random false-positive voxels*. The percentage was again *larger* than any value in the null distribution of overlaps of permuted same-suprathreshold-voxel-number maps (P_{RanOvp} < .0002). This confirmed that the observed intersection *did not occur by chance*.

Figure 5-7 Spatial overlap of group difference maps in GM

Panels A and B show the group difference maps projected on the SPM "glass brain" in three views (for each contrast, left upper is the view looking through x-axis, right upper through y-axis, and left lower through z-axis), in order to provide an overall impression of the spatial distribution. For each panel, the right column is MC – FC comparison (i.e., masculinisation pattern), and the left column is MA – MC comparison (i.e., ASC effect in males, panel A) or FA – FC comparison (i.e., ASC effect in females, panel B). In panel C, columns 1 and 2 (from the left side) are the group difference maps to compare. Columns 3 and 4 are voxel number

counts for the two maps, respectively. Column 5 is the count of intersection (i.e., spatially overlapping) voxels of the two maps. Columns 6 and 7 provide the percentage overlap by dividing column 5 by columns 3 and 4 respectively. Column 8 gives the probability (P_{FPV}) that the observed overlap (columns 6 & 7) is smaller than the overlap of two pure random noise maps. Column 9 gives the probability (P_{RanOvp}) that the observed overlap (columns 6 & 7) is smaller than the overlap of two spatially randomly permuted maps with the same suprathreshold voxel numbers as the group difference maps (columns 3 & 4). A summary percentage overlap was defined as the average of the percentages for each set of comparisons (i.e., MA – MC vs. MC – FC, and FA – FC vs. MC – FC, respectively.) Qualitatively observing panels A and B, one could tell that the two columns did not look alike in panel A, but were more similar in panel B (e.g. the overlap in anterior cingulate areas). Quantitatively it was proven that there was a much larger spatial overlap for the female compared to the male groups (20% vs. 0.13%).



C

Map1	Map2	Map1 voxels	Map2 voxels	Map1 ∩ Map2 voxels	% map1	% map2	P_{FPV}	P_{RanOvp}
MA > MC	MC > FC	24275	83857	**40**	**0.16%**	**0.048%**	**1**	**1**
MC > MA	FC > MC	12642	45483	**30**	**0.24%**	**0.066%**	**1**	**1**
FA > FC	MC > FC	31931	83857	**9465**	**29.64%**	**11.29%**	**<.0002**	**<.0002**
FC > FA	FC > MC	42012	45483	**8672**	**20.64%**	**19.07%**	**<.0002**	**<.0002**

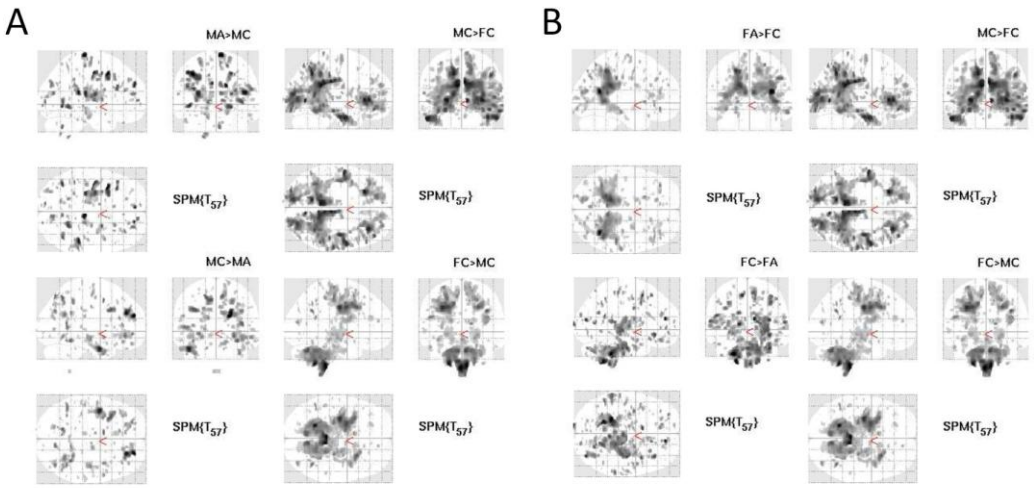
5.3.4.3 Spatial overlap of masculinisation and ASC effect in males and in females: White matter

Similar to what was found in the GM, for WM the MA – MC and MC – FC group difference maps spatially overlapped very little on an average of 0.51% (see Figure 5-8, panels A and C). This intersection was negligible and generated from two non-noise group difference maps ($P_{FPV} = 1$), and was in fact a significant non-overlap ($P_{RanOvp} = 1$).

Again in sharp contrast, the FA – FC and MC – FC group difference maps spatially overlapped to a markedly large extent on an average of 43% (see Figure 5-8, panels B and C). This intersection was not generated by random false-positive voxels ($P_{FPV} < .0002$), and did not occur by chance ($P_{RanOvp} < .0002$).

Figure 5-8 Spatial overlap of group difference maps in WM

The structure of the figure is exactly the same as Figure 5-7, but on WM comparisons. Qualitatively observing panels A and B, one could tell that the two columns did not look alike in panel A, but were obviously similar in panel B (e.g. the overlap on the occipito-parieto-temporal clusters in the upper row, and the cerebellar clusters in the lower row). Quantitatively it was proven that there was a much larger spatial overlap for the female compared to the male groups (43% vs. 0.51%).



C

Map1	Map2	Map1 voxels	Map2 voxels	Map1 ∩ Map2 voxels	% map1	% map2	P_{FPV}	P_{RanOvp}
MA > MC	MC > FC	9868	61650	**171**	**1.73%**	**0.28%**	**1**	**1**
MC > MA	FC > MC	8926	43254	**1**	**0.011%**	**0.002%**	**1**	**1**
FA > FC	MC > FC	34177	61650	**21136**	**61.84%**	**34.28%**	**<.0002**	**<.0002**
FC > FA	FC > MC	22427	43254	**10953**	**48.84%**	**25.32%**	**<.0002**	**<.0002**

5.3.4.4 A validation analysis with larger sample male groups

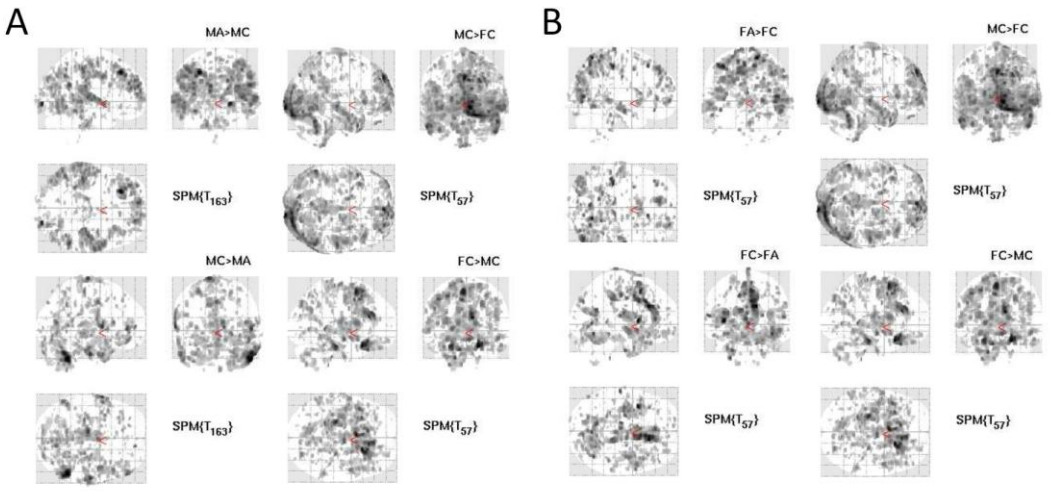
The MA – MC group difference in this sample (N = 30 per group) actually did not survive topological FDR correction. This may be due to an issue of low power. Therefore, the MA – MC comparison was performed again using a much larger

multi-centre sample (N = 84 per group). The increased sample size provides greater power to detect more valid spatial characteristics of the morphometric group differences in males, and helps validate the observed sex-differential pattern regarding how ASC effect overlaps with masculinisation pattern.

The results are illustrated in Figures 5-9 and 5-10. This larger sample revealed spatially broader and more significant MA – MC group differences that survived topological FDR corrections, and the numbers of intersection voxels in both GM and WM increased. However, in all GM and one WM contrasts the intersections were still significant non-overlap. More importantly, all of them were still far smaller compared to what were previously found for the female groups (N = 30 per group). For GM, the average spatial overlap of the larger-sample MA – MC and the original MC – FC group difference maps increased from 0.13% to 1.94%, which was still much smaller than the 20% for the females. For WM, the average spatial overlap for the male maps increased from 0.51% to 4.95%, which was again still much smaller than the 43% for the females.

Figure 5-9 Validation analysis: Spatial overlap of group difference maps in GM, that the male group difference maps were derived from a larger sample (N = 84 per group)

The structure of the figure is exactly the same as Figure 5-7, but the male groups are from a larger sample (panel A). Panel B is exactly the same as that in Figure 5-7. The male overlaps increased but were still substantially smaller than those of the females. Moreover, although for one set of contrasts the overlap was not generated by random false-positive voxels (P_{FPV} = .009), all these new male overlaps were still in fact significant non-overlap (P_{RanOvp} = 1).

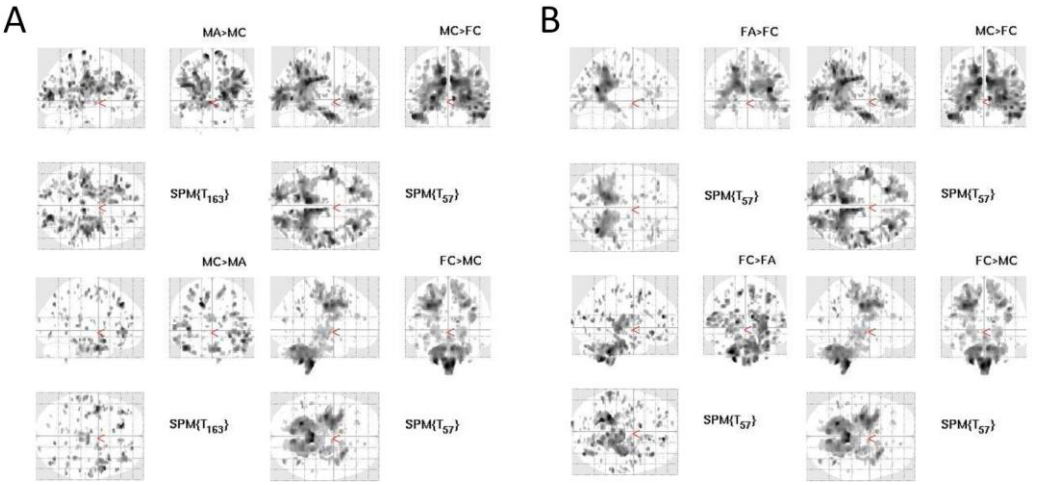


C

Map1	Map2	Map1 voxels	Map2 voxels	Map1 ∩ Map2 voxels	% map1	% map2	P_{FPV}	P_{RanOvp}
MA > MC (N=168)	MC > FC	55287	83857	**1797**	3.25%	2.14%	.009	1
MC > MA (N=168)	FC > MC	39132	45483	**500**	1.28%	1.10%	1	1
FA > FC	MC > FC	31931	83857	**9465**	29.64%	11.29%	<.0002	<.0002
FC > FA	FC > MC	42012	45483	**8672**	20.64%	19.07%	<.0002	<.0002

Figure 5-10 Validation analysis: Spatial overlap of group difference maps in WM, that the male group difference maps were derived from a larger sample (N = 84 per group)

The structure of the figure is exactly the same as Figure 5-8, but the male groups are from a larger sample (panel A). Panel B is exactly the same as that in Figure 5-8. The male percentage overlap increased. All the new male overlaps were not generated by random false-positive voxels (P_{FPV} < .0002), and one new male overlap occurred not by chance (P_{RanOvp} < .0002). However, these new male overlaps were still substantially smaller than those of the females.



C

Map1	Map2	Map1 voxels	Map2 voxels	Map1 ∩ Map2 voxels	% map1	% map2	P_{FPV}	P_{RanOvp}
MA > MC (N=168)	MC > FC	23948	61650	**1823**	7.61%	2.96%	<.0002	1
MC > MA (N=168)	FC > MC	8124	43254	**632**	7.78%	1.46%	<.0002	<.0002
FA > FC	MC > FC	34177	61650	**21136**	61.84%	34.28%	<.0002	<.0002
FC > FA	FC > MC	22427	43254	**10953**	48.84%	25.32%	<.0002	<.0002

5.3.5 Significant local structures and correlations with digit ratio

5.3.5.1 Grey matter

There were no *SSE regions* identified, as reported earlier. For *EMB regions* in females, although there were voxels sprinkling across the brain owing to the nature of the method (i.e., no spatial extent threshold applied to the VBM group difference maps), we found extensive contiguous voxels at the bilateral ACC, SMA and insula showing a pattern of "Female > Male" and "NT > ASC in female". For the pattern "Male > Female" and "ASC > NT in female", contiguous voxels were observed at the bilateral occipito-temporal junction and in the middle portion of middle temporal gyri. See Figure 5-11, panels A and C.

Before examining correlations with digit ratio, the proxy measure of prenatal androgen effect, it is worthy to note that the FA and FC groups were not different on their left (FA: 0.967, SD = 0.0322; FC: 0.975, SD = 0.0287; $t_{(58)} = 1.010$, $p = 0.317$) or right (FA: 0.971, SD = 0.0256; FC: 0.972, SD = 0.0293; $t_{(58)} = 0.171$, $p = 0.865$) 2D:4D ratios.

There was a positive correlation of the left-hand 2D:4D ratio to the average relative GM volume in the "Female > Male (FC > MC)" and "NT > ASC in female (FC > FA)" intersection voxels (red clusters in Figure 5-11; $r = 0.32$, $p = 0.013$, see panel B). This suggests that increases in prenatal androgen effect (i.e., lower 2D:4D ratio) correlates with decreases in the volume of these structures. Analysing by group, the correlation in female controls was $r = 0.52$ ($p = 0.003$), but was not significant in females with ASC ($r = 0.20$, $p = 0.296$). The difference between the two correlations (assessed with Fisher's *r*-to-*z* transform (Fisher, 1921)) was not significant ($Z = 1.36$,

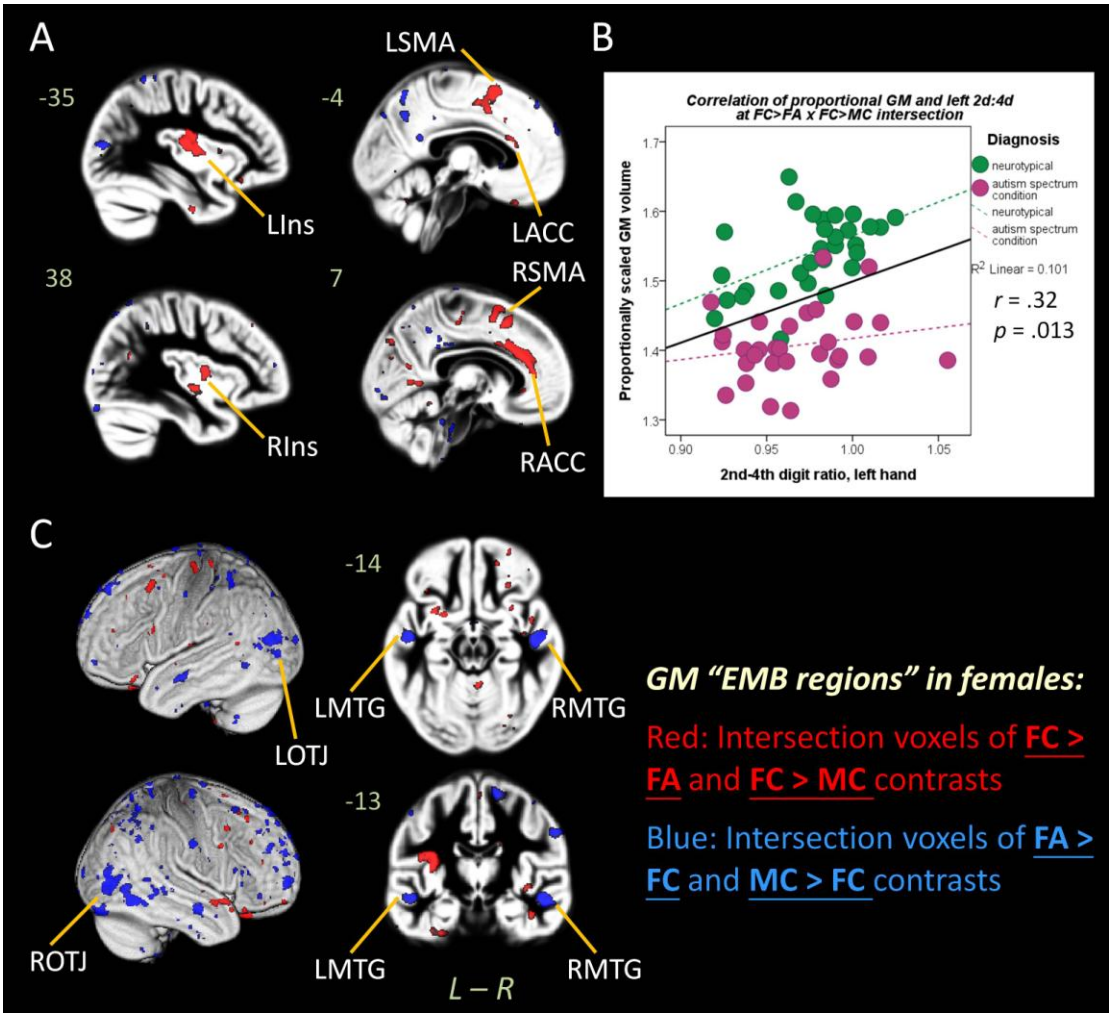
p = 0.17). When examining the contiguous voxels by the main anatomical structures involved (i.e., right and left ACC, SMA and insula), the correlation was significant in two structures: (i) right ACC for the whole group (r = 0.27, p = 0.04), and separately only for FC (r = 0.48, p = 0.007; also for right-hand, r = 0.38, p = 0.04) but not FA (r = -0.002, p = 0.99); and (ii) right SMA for the whole group (r = 0.34, p = 0.007), and separately only for FA (r = 0.43, p = 0.017) but not FC (r = 0.22, p = 0.24). Considering multiple comparisons, the correlations should be viewed conservatively.

No other significant volumetric correlation was found for the right 2D:4D ratio, and no correlation to digit ratio was found for the "Male > Female (MC > FC)" and "ASC > NT in female (FA > FC)" intersection voxels (blue clusters in Figure 5-11). No significant intersection was found in the MA – MC vs. MC – FC overlap analyses so the above analyses were not performed.

Additionally, it is worth noting that when the height threshold was relaxed to $p <$ 0.05 in the previous two-way factorial design VBM, regions involving bilateral insula and superior temporal gyri survived multiple comparison correction as showing interaction with a pattern of FC > FA and MA > MC. The *EMB regions* at bilateral insula (red clusters in Figure 5-11) fell exactly into it. This implied that some of the GM *EMB regions* showed a potential role as *SSE regions* as well.

Figure 5-11 GM "EMB regions" in females and the correlation to 2D:4D ratio

Panel A shows the intersection voxels for FC > FA vs. FC > MC contrasts (in red). Contiguous voxels were found at the bilateral anterior cingulate cortices (LACC, RACC), supplementary motor areas (LSMA, RSMA) and insula (LIns, RIns). Panel B shows positive correlation of their relative volume to the left hand 2D:4D ratio. Panel C shows the intersection voxels for FA > FC vs. MC > FC contrasts (in blue). Contiguous voxels were found bilaterally at the middle portion of middle temporal gyri (LMTG, RMTG) and occipito-temporal junction (LOTJ, ROTJ).



5.3.5.2 White matter

For *EMB regions* in females, there were far less sprinkling voxels but large clusters of contiguous voxels bilaterally at the cerebellum (involving ponto-cerebellar tracts) for the pattern "Female > Male" and "NT > ASC in female", and at the occipito-parieto-temporal junction region for the pattern "Male > Female" and "ASC > NT in female" (involving cingulum, corpus callosum, AF and ILF); see Figure 5-12 panel A, left column. Furthermore, the latter nearly completely overlapped with a region showing significant sex-by-ASC interaction in the two-way factorial design VBM; see Figure 5-12 panel A, right column. These indicate that this *EMB region* concurrently shows statistically significant sex-dependent diagnostic group difference, which means it is also an *SSE region*. Therefore, it reflects *sex-specific extreme male brain* pattern and can be viewed as the "*SSEMB region*".

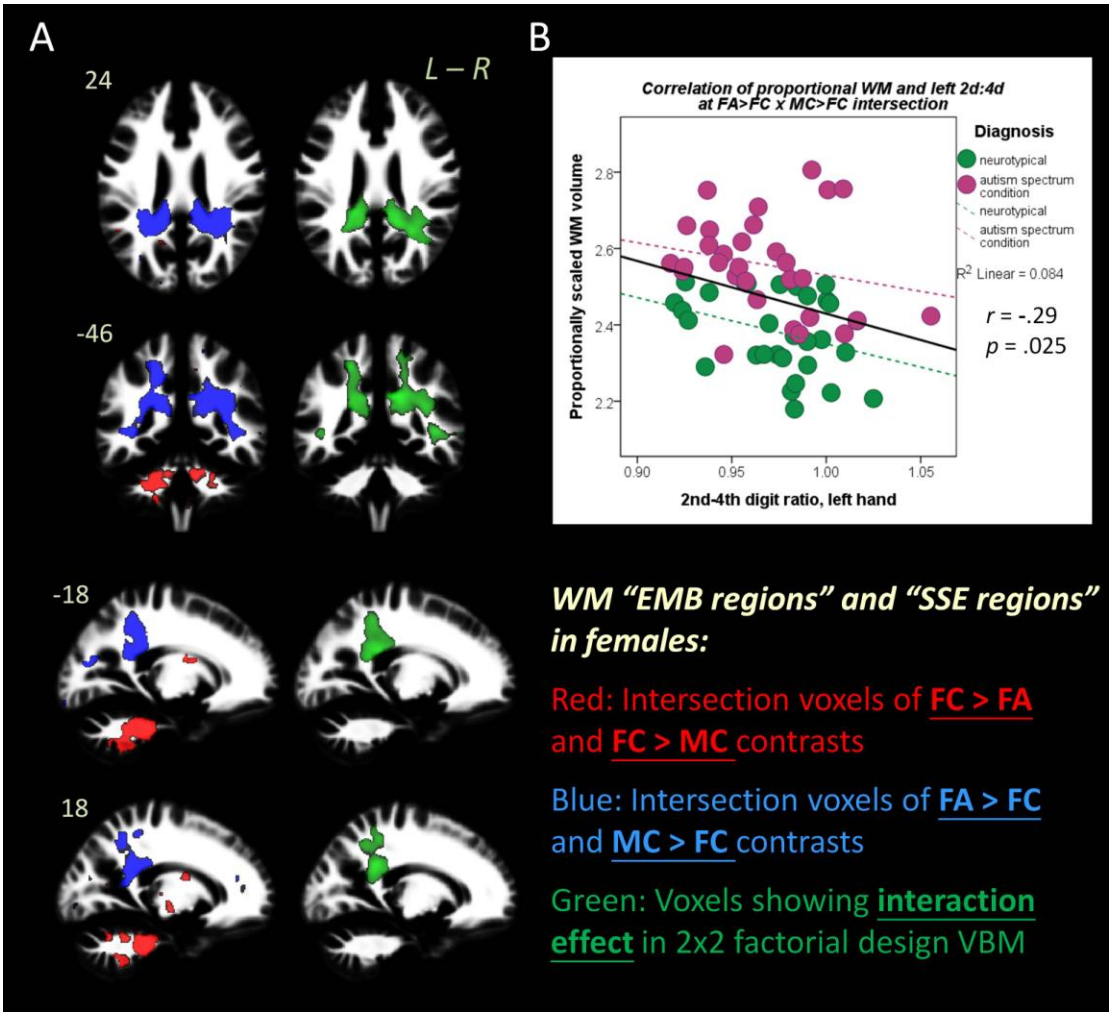
There was a negative correlation between the left-hand 2D:4D ratio and the average relative WM volume in the "Male > Female (MC > FC)" and "ASC > NT in female (FA > FC)" intersection voxels (blue clusters in Figure 5-12; r = -0.29, p = 0.025, see panel B). The implication is that the higher prenatal androgen effect (i.e., lower 2D:4D ratio), the larger volume of these regions. When analysed by group, the correlation became marginally significant in FC (r = -0.34, p = 0.07) and non-significant in FA (r = -0.22, p = 0.25); the two correlations were not statistically different (Z = -0.47, p = 0.64).

No significant volumetric correlation was found for the right 2D:4D ratio, and no correlation to digit ratio was found for the "Female > Male (FC > MC)" and "NT > ASC in female (FC > FA)" intersection voxels (red clusters in Figure 5-12). Once again, no significant intersection was found in the MA – MC vs. MC – FC overlap

analyses so the above analyses were not performed.

Figure 5-12 WM "EMB regions" and "SSE regions" in females and the correlation to 2D:4D ratio

Panel A shows the intersection voxels for FC > FA vs. FC > MC contrasts (red), and FA > FC vs. MC > FC contrasts (blue). The latter replicated the clusters showing a sex-by-ASC interaction in the previous two-way factorial design VBM (SSE regions, green). The majority of the intersecting voxels were contiguous and constituted bilateral clusters: the former (red) involving ponto-cerebellar tracts and the latter (blue) involving cingulum, corpus callosum, AF and ILF. Panel B shows the negative correlation for the relative WM volume of the latter (blue) to the left hand 2D:4D ratio.



5.4 Discussion

The aim of this chapter was to investigate the relationship between sex and ASC at the level of brain morphometry, by testing the three candidate models: OG, SSE and EMB. We showed that the best descriptive model was a combination of the SSE and EMB models (i.e., SSEMB model), that an EMB model applied to females but not to males. Volume of the SSEMB regions also moderately correlated with the proxy index of prenatal androgen exposure effect.

5.4.1 What is the best model: OG, SSE, EMB or SSEMB?

The candidate models (Figures 5-1 and 5-2) were proposed according to both statistical principles and cognitive theories for autism. For WM, given the definite presence of an interaction between sex and ASC, the OG model is rejected and the SSE model supported. For GM, we noticed trend significant interaction, whilst the two main effect maps were mainly (though not completely) spatially distinct. The small overlapping regions of the two main effect maps (Figure 5-5) conform to the EMB-implicated directionality (i.e., Male > Female overlaps with ASC > NT, and Female > Male overlaps with NT > ASC). Therefore, the OG model cannot be rejected but evidence (though weak) supporting the SSE and EMB models also exists. This uncertainty for GM could be due to power issues and studies with a larger sample may provide further validation.

The EMB theory is conceived mainly from observations at the cognitive level and specifically relates to dimensions of empathy and systemizing (Baron-Cohen, 2002; Baron-Cohen, Knickmeyer, et al., 2005). This chapter is the first to test

predictions from the EMB theory at the neural level in terms of brain morphometry. Global brain (GM, WM and CSF) volumes did not follow its predictions. At a more localised level, there were relatively spatially distinct effects of sex and ASC, which does not support a full-EMB pattern (i.e., two main effect maps overlap substantially in the brain) either. However, on examining the spatial overlap separately by sex, an EMB pattern exists to a substantial extent in females but not in males.

Typical sexual dimorphism showed substantial intersection with brain regions different in volume in ASC compared to typical females. This overlap was on average 20% for GM and 43% for WM, both proved not resulting from false-positive voxels and not occurring by chance. Furthermore, the majority of these overlapping voxels were in large spatially contiguous clusters, particularly in the WM. By striking contrast, there was little intersection between typical sexual dimorphism and ASC-control group differences in males in GM by an average of 0.13% but 1.94% in a larger sample, both however reflected significant non-overlap. Similarly for WM, there was little intersection between typical sexual dimorphism and ASC-control group differences in males (0.51% but 4.95% in a larger sample), again reflecting significant non-overlap. Furthermore, these small numbers of intersection voxels scattered across the brain and did not cluster contiguously.

This female-male discrepancy is greater than a 10-fold difference, and only in females the overlaps are all proved to be real and non-random. Current picture therefore supports an *SSEMB model* that the EMB pattern stands out in females but not in males with ASC. This difference between sexes was statistically significant in WM and marginal in GM, that the *EMB regions in females* were also identified as the *SSE regions* by the two-way factorial design VBM.

In sum, for brain morphometry male and female adults with ASC seemed to differ from their same-sex controls differentially (i.e., SSE). Particularly, females with ASC had a pattern of morphometric difference from typical female adults that was substantially similar to masculinisation, which did not occur for males with ASC. This suggests that the EMB theory extends to the brain structural level in a sex-specific fashion (i.e., SSEMB).

5.4.2 How neuroanatomical phenotype informs potential underlying mechanisms

Why is it important to discover the statistical relationship between sex and ASC? As depicted in Chapter 1, observations not only provide characterisation of a condition, but also serve as the basis to generate hypothesis about its emergence. From the observed SSEMB pattern, one speculation is that mechanisms contributing to the brain morphometric features of men and women with ASC may be partially different. For women biological underpinnings that masculinise the brain may contribute to the development of ASC, whereas this may not be explicit for men.

5.4.2.1 Prenatal androgen effect?

One major biological process contributing to brain masculinisation is prenatal androgen effect (Hines, 2005, 2010). The correlation in females for relative volume of the EMB regions to left-hand 2D:4D ratio provides corresponding supports to the idea that prenatal androgen stimulation has a masculinising effect on the brain. Although an indirect index of androgen exposure effect in the womb (Breedlove, 2010; Manning, et al., 2001; Manning, et al., 1998), 2D:4D ratio is the best available proxy that reflects the effect of an early biological process, since it is reported to be fixed

within the first three months after conception and is stable for life (Garn, Burdi, Babler, & Stinson, 1975; Manning, et al., 1998); but note that a recent report shows it might also be influenced by neonatal testosterone (Knickmeyer, Woolson, Hamer, Konneker, & Gilmore, 2011). A further reason for suspecting that prenatal androgens may be at work in the autistic brain comes from the fact that ASC frequently develops within the first year of life both behaviourally (Ozonoff et al., 2010) and with respect to neuroanatomical features (Courchesne, et al., 2003); although evident presentation of ASC after a period of temporary developmental regression later in toddler age does occur at times (Parr et al., 2011; Werner & Dawson, 2005), and seems specific to autism but not other neurodevelopmental disorders (Baird et al., 2008; Pickles et al., 2009). It is also known within typical development, prenatal (foetal) testosterone (fT) level measured via the amniotic fluid correlates to individual differences on a range of cognitive and behavioural features relevant to ASC. For example, fT is inversely correlated with eye contact at 12 months old (Lutchmaya, Baron-Cohen, & Raggatt, 2002), vocabulary at 18 and 24 months (Lutchmaya, Baron-Cohen, & Raggatt, 2001), quality of social relationships at 48 months (Knickmeyer, Baron-Cohen, Raggatt, & Taylor, 2005) and empathy at 48 and 96 months (Chapman et al., 2006; Knickmeyer, Baron-Cohen, Raggatt, Taylor, & Hackett, 2006). fT is positively correlated with restricted/narrow interests at 48 months (Knickmeyer, et al., 2005) and systemizing at 96 months (Auyeung et al., 2006). Finally, fT is also positively related to autistic traits at 18-24 months (Auyeung, Taylor, Hackett, & Baron-Cohen, 2010) and 6-10 years of age (Auyeung, Baron-Cohen, et al., 2009). Though a direct relationship between fT and actual individuals with a diagnosis of ASC has yet to be established, the influence on a range of measures related to the autistic phenotype suggests that it may be a plausible mechanism influencing neurodevelopment in autism (Baron-Cohen, et al.,

2011).

The *SSEMB regions* identified here were those larger (or smaller) in women with ASC than in typical women, and at the same time larger (or smaller) in typical men than in typical women. For one set of these regions in GM (bilateral ACC, SMA and insula) and WM (bilateral posterior cingulum, corpus callosum, AF and ILF) the relative volume correlated with left-hand 2D:4D ratio in typical women, but not in women with ASC. The correlations were in line with the group difference patterns. For the GM regions, they were *smaller* in women with ASC than in typical women, and *smaller* in males than in females. The higher prenatal androgen stimulation, the *smaller* the volume was (mainly at right ACC and SMA). These confirm that this region reflects neuroanatomical feature of ASC, shows sexual dimorphism, and its relative volume correlates to prenatal androgen stimulation. The same (but in an opposite direction) occurs in WM for bilateral posterior cingulum, corpus callosum, AF and ILF. Some of these structures are known to show high density of sex steroid receptors in animals during early development, e.g. ACC (A. S. Clark, MacLusky, & Goldman-Rakic, 1988; Kolb & Stewart, 1991; Pfaff & Keiner, 1973; Shughrue, Stumpf, MacLusky, Zielinski, & Hochberg, 1990; Sibug, Stumpf, Shughrue, Hochberg, & Drews, 1991) and SMA (A. S. Clark, et al., 1988). It is thus plausible that these SSEMB regions are sensitive to prenatal androgen effect, reflected by their size.

5.4.2.2 One step further: Ceiling effect hypothesis and other modulating factors?

Women (but not men) with ASC were "more masculinised" in the SSEMB regions. Correlations with 2D:4D were only significant in typical women but not in women with ASC. One explanation for these is a *ceiling effect*. Although prenatal

androgen may affect the volume of SSEMB regions, explicit correlation is not observed in women with ASC because they may have already been *masculinised to the extreme for females*. A second ceiling effect may underlie the lack of extreme masculinisation in males with ASC. The male foetus is exposed to much higher prenatal androgen stimuli than females (Dawood, 1977; Finegan, Bartleman, & Wong, 1989; Nagamani, McDonough, Ellegood, & Mahesh, 1979). If the brain of typical male has already reached its maximum in terms of masculinisation, it would be difficult to imagine scenarios where males with ASC were more extreme, when they are already at ceiling. Contrast this to females, who are not at ceiling in terms of volume of local structures sensitive to biological masculinising factors, volumetric masculinisation in females with ASC may be easier recognisable than that of males with ASC because females are not limited by being at ceiling.

It is worth noting that although previous studies have reported lower 2D:4D ratio in children with ASC (Manning, et al., 2001; Noipayak, 2009) and in girls with psychiatric conditions who have higher autistic features (De Bruin, De Nijs, Verheij, Verhagen, & Ferdinand, 2009), women with and without ASC in this study did not differ in their 2D:4D ratio. If this lack of an effect is not due to a lack of power, it would suggest that masculinised brain morphometry in females with ASC is not directly corresponding to prenatal androgen stimulation. Instead, other modulating factors may be affecting the masculinisation pattern, e.g. sensitivity to the consequences of androgen exposure. Similarly, a lack of morphometric masculinisation of the brains in males with ASC does not directly rule out the potential role of prenatal androgen in the emergence of ASC in males. There may be other modulating factors as well. For example, experiential effects of stress,

co-morbid depression and anxiety are generally related to shrinkage and atrophy of the brain (Duman, 2009). It may also be that prenatal androgen effects act on other neural properties (e.g. neural wiring or remodelling) than volumetric change itself (Sullivan & Moenter, 2004). Thus, the lack of an effect for males in terms of volume may be due to other factors masking any true effect of extreme masculinisation.

5.4.2.3 Relevance to other studies

The SSEMB pattern matches up well with a recent report on sex-specific serum biomarkers for ASC (Schwarz, et al., 2010). Serum biomarkers in male adults with ASC include mainly cytokines and inflammatory molecules, whereas for female adults with ASC the biomarkers consist of mainly androgen-related, growth hormone and insulin-related molecules. The same study also found increased serum free testosterone only in females but not males with ASC. Within this same cohort, both male and female adults with ASC have elevated levels of androstenedione (a precursor to testosterone) but the effect size is larger in females (Ruta, Ingudomnukul, Taylor, Chakrabarti, & Baron-Cohen, 2011). Along with the current results, these suggest that differential biological processes are at work in males and females with ASC, and hormonal (especially androgen) factors may be more impactful in females. As pointed out in Chapter 1, a cross-sectional study on adults with ASC only observes the final product of primary characteristics plus developmental changes, involving complex (epi)genetic, hormonal, biological and neurobiological pathways. Other study designs (e.g. longitudinal follow-up, intervention design) that focus on early development are required to clarify and substantiate whether these observations reflect core mechanisms for ASC or compensatory processes related to living an entire life with ASC.

5.4.2.4 Weakness of the SSEMB-prenatal androgen hypothesis

The SSEMB model best describes how sex and ASC are related in terms of brain morphometry. The potential association of SSEMB region size to prenatal androgen exposure further provides testable hypotheses regarding sex-specific roles of prenatal hormonal effects for males and females with ASC. It also provides a framework to examine whether there is a ceiling effect of prenatal androgen exposure and how it arises, and whether there are other modulating factors. However this hypothesis has certain weakness. First, masculinisation does not explain all ASC effects in females (i.e., the map overlaps were 20% in GM and 43% in WM, but not 100%). Second, 2D:4D ratio only indirectly explains a small amount of variance of prenatal androgen exposure effect (Breedlove, 2010). Third, the correlation of SSEMB regional volume to 2D:4D ratio should be interpreted conservatively because it only occurred for the left hand and half of the SSEMB regions, the effect sizes were moderate, and the significance level was not corrected for multiple comparisons. Lastly, the *brain organisational theory of prenatal androgen* itself is not without its critics and should not be oversimplified, due to inconsistent findings, research methodological issues and alternative explanations (Jordan-Young, 2010; Valla & Ceci, 2011). To test this *SSEMB-prenatal androgen hypothesis*, future studies should investigate if prenatal androgen effects have differential neuroanatomical influences on boys and girls longitudinally, and if prenatal androgen effects predict the diagnosis of ASC differentially for boys and girls. It is also important to delineate if there are other factors modulating prenatal androgen effects on brain anatomy.

We found a correlation with the left but not right hand 2D:4D ratio to SSEMB region volumes. Although this is in line with an earlier report on girls showing a

correlation between left (but not right) hand 2D:4D ratio and autistic symptoms (De Bruin, et al., 2009), other two studies on male-predominant samples found the correlations for both hands (Noipayak, 2009) or the average of both hands (Manning, et al., 2001). Previous studies report that right hand 2D:4D ratio is more responsive to prenatal androgen effects (Breedlove, 2010; Lutchmaya, et al., 2004), for unknown reason. The explanation (e.g., whether either hand reflects the effect better for either sex) behind this partial non-replication awaits clarification.

5.4.3 Additional findings

A few additional findings deserve attention. First, we found no difference in GM, WM and CSF volumes between adults with and without ASC, in both males and females. This is in agreement with prior studies with adolescents (Aylward, Minshew, Field, Sparks, & Singh, 2002) and adults with ASC (Hallahan, et al., 2009; McAlonan, et al., 2002; Toal, et al., 2010), as well as with women with ASC (M. C. Craig, et al., 2007). It lends support to the model of early brain overgrowth with later regression and normalisation of brain volume by adulthood (Courchesne, et al., 2011; Courchesne, et al., 2007). The added value here is to demonstrate that this is equally the case for males and females.

The other crucial finding is that by applying the same processing and statistical modelling in equal-sized groups, ASC-control morphometric differences survived multiple comparison correction only in females. This suggests that the effect size of ASC-control group difference is larger in females than in males. Much larger sample size is required to detect group difference in males. This plausible sex difference in the effect size of atypical neuroanatomical feature of ASC generates new hypotheses

(e.g., if females with ASC are more homogenous than males with ASC) and warrants further investigation.

5.4.4 Limitations and future directions

Since all analyses were conducted using VBM, they are prone to the same limitations depicted in Chapter 4. Applying the analytic strategies in this chapter to other structural measures (e.g. surface-based morphometry) will extend current findings.

Second, there are sex differences in the developmental trajectories of brain growth from childhood to early adulthood (Giedd, et al., 1999; Lenroot, et al., 2007; Shaw, et al., 2008; Tiemeier, et al., 2010). It is plausible the neuroanatomical aging process after early adulthood is also sexually dimorphic. In addition, it has been shown that cortical neuroanatomy in male adults with ASC may have a different aging trajectory compared to typical male adults (Raznahan, Toro, et al., 2010). The present study did not cover potential complex interactions amongst aging, sex and ASC, which should be a next step of investigation using larger sample.

Third, another unaddressed question is why the SSEMB pattern is more prominent in WM than in GM. WM and GM volumes represent different underlying biological substrates and show different growth trajectories (Giedd & Rapoport, 2010). Future work addressing how different biological mechanisms influence WM and GM will help clarify the biological implications of the SSEMB pattern.

Fourth, as discussed in Chapter 4, the female groups were not matched on oral contraceptive use, nor on the phase of their menstruation cycle. This type of cyclic

change in hormonal status is potentially not that evident in males. In future work it will be important to examine how *current* hormonal status relates to brain measures. We have collected saliva sample for all participants and a variety of hormones (particularly sex steroids) will be analysed shortly.

Lastly, more women with ASC than typical women received medication for mood and/or anxiety disorders at the time of study. The same occurred in men, but overall fewer males than females were medicated (MC: 0/30, MA: 6/30, FC: 3/30, FA: 14/30). Although high comorbidity in adults with ASC is common (Hofvander, et al., 2009; Lugnegard, et al., 2011; Tantam, 2000), further work should address how comorbidity (and medication) affects neuroanatomy in ASC.

Chapter 6

Brain Oscillations in ASC:

Differential Complexity in Males and Females

6.1 General Introduction

6.1.1 Resting-state functional MRI

The previous chapter has investigated brain features for males and females, with and without ASC in terms of *structure*. Another aspect to understand the brain is its *functional organisation*. Recent advances in the technique and analytical strategies (Fornito & Bullmore, 2010) of *resting-state*[7] functional MRI (fMRI) provide another window apart from the cognition-oriented task-evoked fMRI paradigm. Resting-state fMRI provides multivariate dataset sampling *intrinsic functional organisation* of the brain (Greicius, 2008), which can be reliably and consistently mapped across time (Shehzad et al., 2009), individuals (Damoiseaux et al., 2006), species (Rilling et al., 2007; Vincent et al., 2007) and development (Fair et al., 2008; Fair et al., 2009; Fair et al., 2007; Fransson et al., 2007; Gao et al., 2009), and is relatively invariant to scanning conditions (e.g., eyes closed or open, sleep or awake, light or heavy sedation) (Fox & Raichle, 2007). Furthermore, multiple spatiotemporally independent neural circuits (De Luca, Beckmann, De Stefano, Matthews, & Smith, 2006; Fox et al., 2005) can be simultaneously identified and show close correspondence with underlying structural connectivity (Greicius, Supekar, Menon, & Dougherty, 2009; Honey et al., 2009;

[7] This has been criticised regarding the difficulty in interpreting what a "resting state" really is (Morcom & Fletcher, 2007). Here we treat resting-state fMRI simply as a tool sampling brain activities during a condition that no explicit cognitive tasks are assigned to the participant, but we do not imply there are no ongoing cognitive activities. Conceptually resting-state fMRI can be viewed as parallel to the conventional resting electroencephalography (EEG), but records spontaneous brain activities (oscillations) in lower frequency range.

Skudlarski et al., 2008; van den Heuvel, Mandl, Kahn, & Hulshoff Pol, 2009) and functional networks activated by cognitive tasks (Smith et al., 2009).

Much of what is known about intrinsic functional organisation of the brain during resting-state fMRI rests on the inferences from *functional connectivity*. Whilst connectivity approaches are useful in assaying the relationships *between regions*, their limitation is the lack of direct indications on *within-region* abnormalities across time. In contrast, *regionally specific measures* of resting-state fMRI recognise that complex neural organisation can be characterised in multiple ways other than through functional connectivity. Amongst the limited number of validated measures (Fornito & Bullmore, 2010), one parsimonious way to estimate the complexity of the spontaneous fluctuation of blood-oxygen-level-dependent (BOLD) signal (i.e., brain oscillations) is by its *fractal geometry*.

6.1.2 An illustration to fractality and fractal parameters

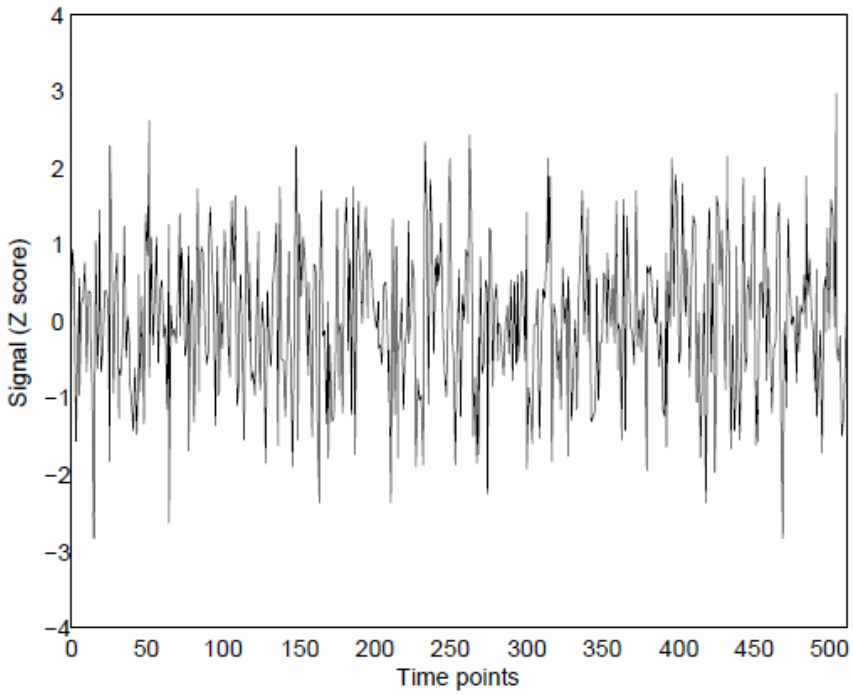
Fractality is found extensively in complex biological, natural and social systems (Strogatz, 2001) and is indicative of development through repeated feedback mechanisms based on simple scaling rules (Gribbin, 2005). Simply put, a fractal system "looks approximately the same" on many scales of space and time. In fractal geometry, the fractal (Hausdorff) dimension, *FD*, characterises the complexity. For example, in fMRI time series, the fractal dimension lies in $1 < FD < 2$, that has a *topological dimension* of 1 (it is a Euclidean *line*) and an *embedding dimension* of 2 (it is located in a Euclidean *plane*). Looking at a time series of random (white) noise that has $FD = 1.5$ (equal to a Hurst exponent = 0.5, see text below and Figure 6-1), one finds the line more irregular than a Euclidean line, but not rough enough to occupy the entire plane. For a

fractal process, the closer its *FD* is to the embedding dimension, the greater geometric complexity it possesses (Bullmore et al., 2009). The presence of three defining properties imply that the spontaneous BOLD signal fluctuation in fMRI is *fractal* (Herman, Kocsis, & Eke, 2009; Mandelbrot, 1977), can be subjected to fractal analysis and is characterisable on a regionally specific manner: (i) a power spectrum that follows a $1/f^{\gamma}$ distribution (i.e., has disproportionate power in the low frequencies, thus showing a *power-law scaling relationship*); (ii) correlation over time (i.e., autocorrelation and *self-similarity*); and (iii) a *scale invariant* property (Bullmore et al., 2003; Bullmore et al., 2004; Kitzbichler, Smith, Christensen, & Bullmore, 2009; Zarahn, Aguirre, & D'Esposito, 1997).

Applying fractal analysis to resting-state fMRI, spontaneous BOLD signal fluctuations can be modelled by *fractional Gaussian noise* (fGn) and parameterised by a parsimonious fractal descriptor, e.g. a wavelet-based estimator of the *Hurst exponent (H)*. In brief, *H* of a time series is estimated across the scales of the wavelet transform and is simply related to the fractal dimension, *FD*, by the relation $FD = 2 - H$, and to the spectral exponent, $\gamma = 1 - 2H$, of the spectral density function of the signal (i.e., time series) (Bullmore, et al., 2004; Maxim et al., 2005). If $0 \leq H < 0.5$, the signal auto-covariance is negative and the signal is anti-correlated; if $H = 0.5$ it is a white (random) Gaussian noise; if $0.5 < H \leq 1$, the auto-covariance is positive and the signal has long-memory or positive autocorrelations over long time lags, and adequately models fMRI time-series. As such, *H* is a parsimonious variable summarising the scaling behaviour of the signal across wavelet bands, from low frequency BOLD fluctuations upon which so-called resting-state networks are derived, to higher frequencies associated with task-related activation; note the lowest (signal drift) and

highest (white noise) wavelet bands are ignored in the estimation. Thus, unlike much of the literature on resting-state fMRI in which signals are low-pass filtered prior to analysis, *H* represents contributions from both trait- and state-dependent factors (Fox, et al., 2005) and their possible interaction. Furthermore, the voxel-wise estimation of *H* allows commonly applied mass-univariate testing. We are therefore motivated by the convenience of resting-state fMRI acquisition in clinical populations and the use of *H* as an omnibus statistic to identifying correlates of neuropsychiatric conditions in resting BOLD time-series as a precursor to regional scrutiny without more speculative methods.

Figure 6-1 A time series of white (uncorrelated random) noise with FD = 1.5 (H = 0.5)



6.1.3 Plan for this chapter

The use of *H* in summarising the local property of neuronal oscillations and intrinsic organisation, though appealing and parsimonious, has only been demonstrated in limited conditions (yet proved sensitive) for localising differences in resting-state dynamics as a function of age, psycho-active drug administration (Wink, Bernard, Salvador, Bullmore, & Suckling, 2006) and mild Alzheimer's disease (Maxim, et al., 2005). It is thus crucial to first investigate if it is sensitive in capturing atypical brain regions in ASC. In Study 1 fractal analysis was applied to male adults with and without ASC, as a *proof of method*, since there are a lot more literatures reporting atypical brain regions in males than in females with ASC. Study 1 showed that *H* well captured group difference in brain regions reported atypical in ASC in past literatures. Therefore in Study 2, females with and without ASC were analysed together with the male groups in a two-way factorial design to investigate the relationship between sex and ASC at the level of brain functional organisation in terms of oscillations.

6.2 Study 1: A Proof of Method in Males- A Shift to Randomness of Brain Oscillations in Male Adults with ASC

6.2.1 Introduction

Autism spectrum conditions (ASC) are characterised by impairments in social communicative development, alongside repetitive stereotyped behaviour and/or restricted interests. Plausible underpinning neurobiological mechanisms involve limbic system (Baron-Cohen et al., 2000; Bauman & Kemper, 2005), mentalizing circuit (Baron-Cohen et al., 1994; U. Frith, 2001), prenatal hormone (Baron-Cohen, 2002), early brain overgrowth and structural abnormalities (Amaral, et al., 2008; Courchesne, et al., 2007), cerebral minicolumnopathy (Casanova et al., 2006), aberrant neural connectivity (Baron-Cohen & Belmonte, 2005; Belmonte, et al., 2004; Just, et al., 2004) and synchronisation (Brock, Brown, Boucher, & Rippon, 2002; Uhlhaas & Singer, 2006). Atypicality in specific components of systems has also been demonstrated, for instance in the social brain (Baron-Cohen & Belmonte, 2005; Baron-Cohen, et al., 2000), cortico-striatal system (Amaral, et al., 2008) and the 'default network' (Kennedy & Courchesne, 2008; Monk et al., 2009; Weng et al., 2010).

Here we further investigate whether neural systems are dynamically atypical in people with ASC. Rather than measuring differences between ASC and control groups in terms of activation by an experimental task, we have measured differences in the complexity of endogenous, low frequency neurophysiological processes, using fMRI in a resting-state (Bullmore, et al., 2009; Bullmore, et al., 2004; Maxim, et al., 2005). Previous studies of autism using resting-state fMRI have shown relatively lower

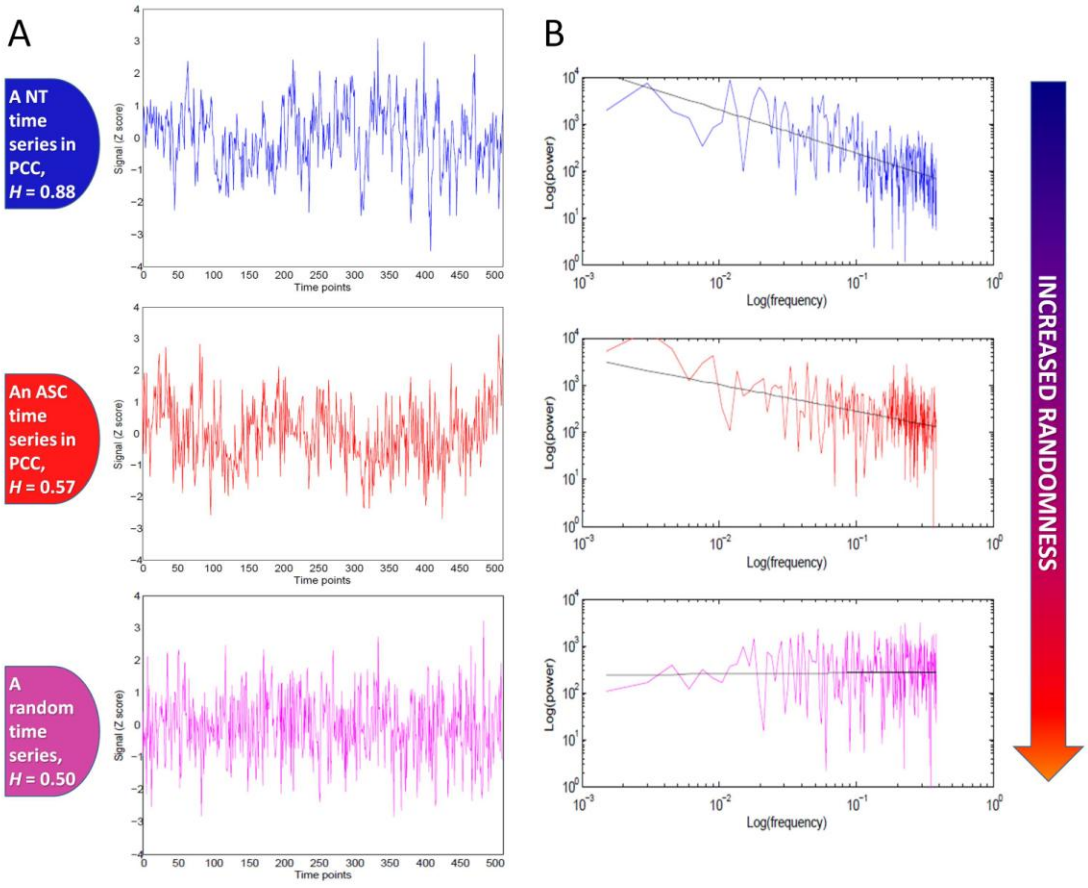
functional connectivity within the default network (Kennedy & Courchesne, 2008; Monk, et al., 2009; Weng, et al., 2010) and right-dominant altered 'regional homogeneity' (Paakki et al., 2010). However, no prior studies have investigated autism-related change in the complexity of resting-state fMRI time series.

As described in the general introduction, fractal scaling parameters, such as the fractal dimension (*FD*), the spectral exponent or the Hurst exponent (*H*), can be used to define where an irregular process in space or time is located on the continuum of processes from randomness to Euclidean order. Changes in fractal scaling of physiological time series have been observed as a corollary of disease or aging in the human brain (Maxim, et al., 2005; Rasouli et al., 2006; Wink, et al., 2006) and heart (Beckers, Verheyden, Couckuyt, & Aubert, 2006; Lin et al., 2001), suggesting that such metrics could serve as physiological indicators of medical conditions. Studies of electrocardiographic (ECG) time series have shown that healthy cardiac dynamics have fractal scaling indicative of high complexity processes, which become more regular in association with end-stage heart disease and to a lesser extent in normal aging (Beckers, Verheyden, & Aubert, 2006; Beckers, Verheyden, Couckuyt, et al., 2006; Lin, et al., 2001). Fractal scaling has also been measured in brain electrophysiological (Bullmore et al., 1994; Linkenkaer-Hansen, Nikouline, Palva, & Ilmoniemi, 2001) and fMRI time series (Maxim, et al., 2005; Wink, et al., 2006; Wink, Bullmore, Barnes, Bernard, & Suckling, 2008). Alzheimer's disease has been associated with a shift to higher *H* indicative of more regular/persistent dynamics (Maxim, et al., 2005). Smaller increases in *H* are associated with normal aging and a single dose of muscarinic receptor antagonist (Wink, et al., 2006). To illustrate the time series properties captured by changes in *H*, Figure 6-2 illustrates two fMRI time series sampled from posterior

cingulate cortex, with H = 0.88 and 0.57, as well as a random time series with H = 0.5.

Figure 6-2 Illustrative fMRI and random time series demonstrating the concept of 'shift-to-randomness'

Panel A shows normalised fMRI time series from a voxel in posterior cingulate cortex (PCC, MNI coordinate: -4, -36, 30) from a neurotypical (NT) (upper, blue, H = 0.88) and an ASC participant (middle, red, H = 0.57), as well as a simulated random ("white Gaussian noise") time series (lower, pink, H = 0.50). Both fMRI time series exhibit self-similarity, but the one from the ASC participant shows less persistence; i.e., it is more similar to the random time series. Panel B illustrates the log-log plot of power spectrum for the three time series. For the NT time series, power attenuates as frequency increases (slope < 0); for the random time series, all frequencies are present with equal power in the spectrum (slope = 0); the ASC time series shows an intermediate slope between the NT and random time series, indicating a 'shift-to-randomness'.



In this context, we measured the complexity of resting-state fMRI dynamics in the brains of 30 men with ASC and 33 age- and IQ-matched neurotypical men. We hypothesised that there would be abnormalities of *H* in people with ASC, especially in brain regions known to be involved in autism.

6.2.2 Methods

6.2.2.1 Sample

Participants were recruited as part of the MRC Autism Imaging Multicentre Study (AIMS) project between July 2007 and November 2008. The inclusion criteria for the ASC group were being male, aged over 18 years, right-handed, average intelligence (IQ >= 70), and a clinical diagnosis of autistic disorder or Asperger syndrome based on DSM-IV (American Psychiatric Association, 2000) or ICD-10 criteria (World Health Organization, 1992). Diagnoses of autism were confirmed for 30 ASC participants using the Autism Diagnostic Interview-Revised (ADI-R) (Lord, et al., 1994). An additional three participants who scored one point below cut-off on the repetitive stereotyped behaviour domain of the ADI-R were also included since they met Autism Diagnostic Observation Schedule (ADOS) (Lord, et al., 2000) criteria for 'autism spectrum' and were diagnosed by experienced clinicians.

Neurotypical (NT) participants were recruited through local advertisements and satisfied the same inclusion criteria as the ASC group, except that they did not have an ASC themselves or in their family history. Exclusion criteria for both groups included current or historical psychotic disorders, substance-use disorders, medical disorders associated with autism (e.g. tuberous sclerosis, fragile X syndrome), intellectual disability, epilepsy, hyperkinetic disorder and Tourette's syndrome. Six ASC

participants reported antidepressant use, whereas none of the NT participants did. None of the ASC or NT participants received antipsychotic medication.

Data from three ASC participants were excluded (two due to incomplete brain coverage during scanning and one due to an incidental neuroradiological diagnosis of agenesis of corpus callosum), leaving 30 ASC participants for subsequent analysis; see Table 6-1.

6.2.2.2 Behavioural measures

All participants were assessed using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) which measures verbal, performance and full-scale IQ, along with a series of self-report and neuropsychological assessments (details given in Chapters 2 and 3). All ASC participants were assessed by the ADI-R and ADOS.

6.2.2.3 Resting-state fMRI data acquisition

Functional MRI data were acquired using a 3T GE Signa scanner (General Electric Medical Systems, Milwaukee, WI) at the Magnetic Resonance Imaging and Spectroscopy Unit, University of Cambridge, UK. Participants were asked to lie quietly in the scanner awake with eyes closed for 13 min 39 sec during sequential acquisition of 625 whole-brain T2*-weighted echo planar image volumes with the following parameters: relaxation time (TR) = 1302 ms; echo time (TE) = 30 ms; flip angle = 70^{o}; matrix size = 64 x 64; field of view = 24 cm; 22 AC-PC aligned slices per image volume; 4 mm axial slice thickness; 1 mm slice gap. The first 113 time points were discarded, leaving 512 (2^{9}) images analysed by the discrete wavelet transform for estimation of the Hurst exponent.

6.2.2.4 Resting-state fMRI data pre-processing and time series modelling

Following geometric motion correction, slice timing correction and global image rescaling (Suckling et al., 2006), volumes were spatially smoothed with a two-dimensional Gaussian kernel sized 4.4mm full width at half maximum. The Hurst exponent was estimated at each intra-cerebral voxel using a maximum likelihood estimator in the wavelet domain (Maxim, et al., 2005; Wink, et al., 2008); see Appendix for further information. All preprocessing and time series analysis steps (including the estimation of *H*) were implemented using the CamBA software library (Brain Mapping Unit, Department of Psychiatry, University of Cambridge, Cambridge, UK, http://www-bmu.psychiatry.cam.ac.uk/software/).

The motion-corrected image volumes and maps of *H* in each individual's native space were co-registered in the standard Montreal Neurological Institute (MNI) space by applying the affine transformation parameters previously derived by registration of each individual's time-averaged fMRI data to the EPI template image (http://www.fil.ion.ucl.ac.uk/spm/).

6.2.2.5 Regional parcellation

Each Hurst map was parcellated into a number of different regions by mapping the data onto three sets of regions-of-interest (ROIs) defined independently.

(i) Regions repeatedly reported to be structurally or cerebrovascularly abnormal in autism: These were chosen from abnormal regions highlighted throughout the literature on structural MRI and perfusion-weighted MRI in ASC, including medial frontal structures (Abell, et al., 1999; Ke, et al., 2008; McAlonan et al., 2005; Rojas, et al.,

2006; Waiter, et al., 2004), cingulate cortices (Haznedar et al., 1997; Kwon, et al., 2004; McAlonan, et al., 2005; Thakkar et al., 2008), left pars opercularis (Hadjikhani, Joseph, Snyder, & Tager-Flusberg, 2006; Nordahl et al., 2007), parietal structures (Brieber, et al., 2007; Ke, et al., 2008; McAlonan, et al., 2008; Ryu et al., 1999), temporal structures (Brieber, et al., 2007; Gendry Meresse et al., 2005; Ke, et al., 2008; Kwon, et al., 2004; McAlonan, et al., 2005; Neeley et al., 2007; Ohnishi et al., 2000; Rojas, et al., 2006; Salmond et al., 2005; Salmond, Vargha-Khadem, Gadian, de Haan, & Baldeweg, 2007; Waiter, et al., 2004; Zilbovicius et al., 2000), amygdala (Amaral, et al., 2008; Baron-Cohen, et al., 2000; Mosconi et al., 2009; Munson et al., 2006; Schumann et al., 2004), insula (Hadjikhani et al., 2009; Ohnishi, et al., 2000; Salmond, et al., 2007), caudate (Haznedar et al., 2006; Hollander et al., 2005; McAlonan, et al., 2005; McAlonan, et al., 2008; Rojas, et al., 2006; Stanfield, et al., 2008) and thalamus (Hardan et al., 2006; Hardan et al., 2008; Haznedar, et al., 2006); see Appendix Table 1. The cerebellum was not examined as in some participants its most inferior part was not fully covered in the images. Several "control regions" reported to be intact in ASC were selected for comparison, including the primary sensory, motor and visual cortices. These ROIs were created according to the Automatic Anatomical Labelling (AAL) template (Tzourio-Mazoyer et al., 2002).

(ii) Social brain regions identified to be consistently under-activated in previous fMRI studies of social cognition in autism: These were generated from a set of peak coordinates from quantitative meta-analyses of all fMRI studies

of social task-activations reported to date on autism; see Figure 6-4 and Table 6-2. They were selected from the peaks of hypoactivations (Controls > ASC) across the entire social cognitive literature (Lombardo, Baron-Cohen, Belmonte, & Chakrabarti, 2009). For each peak coordinate the ROI was constructed as a spherical binary mask 10 mm in diameter using the *fslmaths* utility in the FSL 4.1 software library (Analysis Group, FMRIB, Oxford, UK). Hyperactive regions (ASC>Controls) were not examined due to ambiguity across the literature with regards to studies observing hyperactivations as either indicative of deficits (e.g., compensatory recruitment or increased neural effort) or strengths (e.g., increased ease of processing, heightened recruitment because of cognitive strengths) in ASC.

(iii) Non-social brain regions identified to be consistently under-activated in previous fMRI studies of non-social cognition in autism: These were generated by the same procedure as above; see Figure 6-4 and Table 6-2. They were selected from the peaks of hypoactivations (Controls > ASC) across the entire non-social cognitive literature (Lombardo, et al., 2009).

6.2.2.6 Statistical analysis

The main focus was tests of between-group difference in *H* at global, regional and voxel scales. All voxel-level analyses were conducted within a grey matter mask to restrict comparisons to grey matter regions. We also tested (at voxel level) for correlations between *H* and measures of autistic symptom severity.

(i) Between-group comparisons: Separate general linear models (GLM) were

regressed onto global, regional (defined by the ROIs) and voxel estimates of *H*, with group as the independent variable. The standardised regression parameter for group was tested for statistical significance by parametric and non-parametric procedures. For voxel statistics, we used a permutation test which adjusted the probability threshold for statistical significance of multiple comparisons by controlling the expected number of false positive cluster-level tests to be less than one per map (equivalent $p = 0.0027$, two-tailed) (Bullmore et al., 1999; Suckling & Bullmore, 2004; Suckling, et al., 2006).

(ii) Social versus non-social regional effects: A two-way analysis of variance model was fit to the average regional *H* statistics generated by the social/non-social ROIs, comprising main effects of group and social/nonsocial subsets, and the group-by-regional subset interaction, indicating the extent to which group effects were differently expressed in social versus non-social ROIs.

(iii) Correlations with measures of autistic symptom severity: For this we only analysed data from the ASC group (N=30). A GLM was regressed to voxel estimates of *H* with autistic symptoms (including social interaction, communication and repetitive/stereotyped behaviours scores in the ADI-R, and social-communication and stereotyped behaviour and restricted interest scores in the ADOS) as the independent variable and global mean *H* as a covariate. We used the same permutation test as above and applied a less stringent critical value of 3 error clusters per image (equivalent $p = 0.0059$, two-tailed), which was a speculative analysis. Mean *H* was

extracted for each significant cluster for visual representation of the correlation to symptom scores, for which Pearson's correlation was calculated.

Voxel level permutation tests were implemented by the CamBA software. Regional and global level analyses were conducted in SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using independent sample *t* tests and Pearson's correlation. Parametric tests were adopted as all extracted *H* values from the ROIs were normally distributed, as evidenced by non-significance results in normality tests (i.e., Kolmogorov–Smirnov test).

6.2.3 Results

6.2.3.1 Demographics and behavioural characteristics

There were no significant group differences in age or intelligence. The ASC group scored significantly higher on the AQ (Baron-Cohen, Wheelwright, Skinner, et al., 2001), consistent with their diagnosis; see Table 6-1.

Table 6-1 Sample demographics and clinical assessments

	ASC (N=30)		NT (N=33)		Statistics	
	Mean	SD or *range*	Mean	SD	*t* score or *U*	*p*
Demographics						
Age (Years)	26.9	7.4	28.4	6.1	0.87	0.39
Full IQ	112.6	15.9	116.3	11.6	1.05	0.30
Verbal IQ	111.5	14.9	110.8	12.0	-0.21	0.84
Performance IQ[#]	111.2	16.4	118.5	11.4	*366*	0.08
Autistic traits / diagnoses						
AQ	32.8	8.07	15.2	6.86	-9.32	<0.001
ADI-R						
Social	18.2	*10 – 27*				
Communication	15.4	*8 – 25*				
RSB	5.6	*2 – 11*				
Delay	2.8	*1 – 5*				
ADOS-SC	8.3	*1 – 17*				

[#]: Non-normally distributed: Kolmogorov-Smirnov test of normality p = .002, Shapiro-Wilk test of normality p = .003; therefore non-parametric Mann-Whitney test was used for group comparison here for performance IQ.

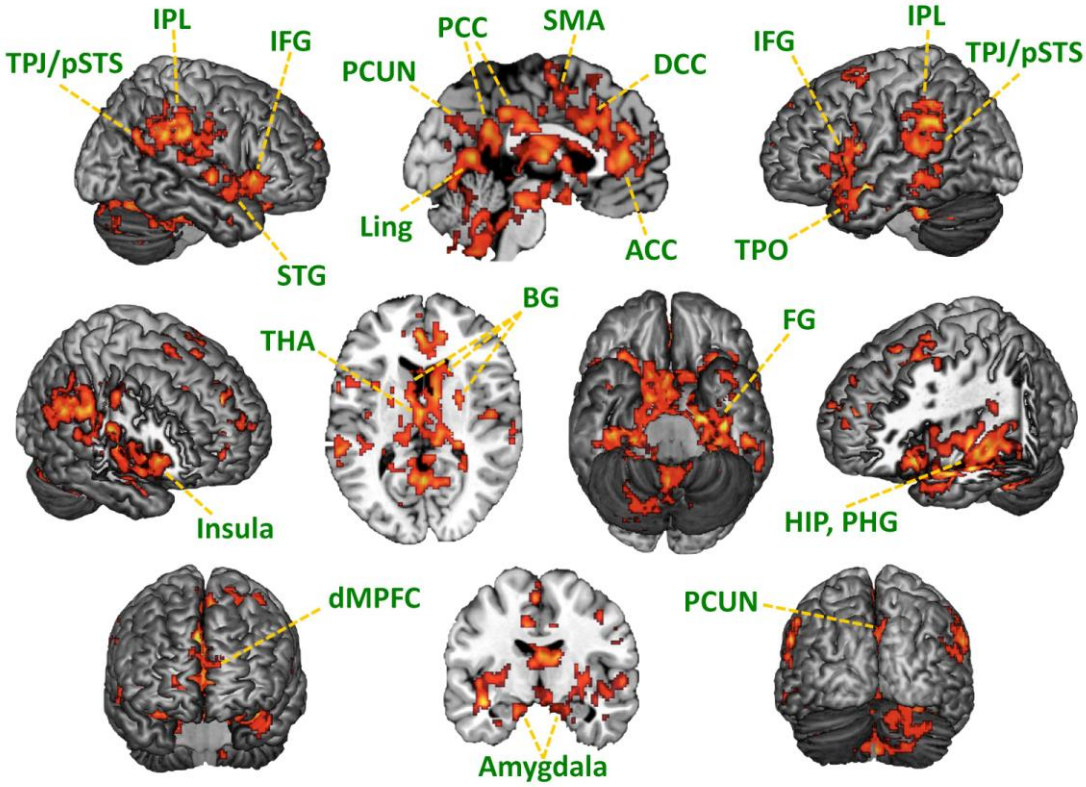
ASC = autism spectrum conditions; SD = standard deviation; U = Mann-Whitney U; IQ = Intelligence Quotient; AQ = Autistic Spectrum Quotient; ADI-R = Autism Diagnostic Interview-Revised; Social = total score in diagnostic algorithm for "Qualitative Abnormalities in Reciprocal Social Interaction", cut-off = 10; Communication = total score in diagnostic algorithm for "Qualitative Abnormalities in Communication", cut-off = 8; RSB = total score in diagnostic algorithm for "Restricted, Repetitive and Stereotyped Patterns of Behaviour", cut-off = 3; Delay = total score in diagnostic algorithm for "Abnormality of Development Evident At or Before 36 Months", cut-off = 1; ADOS-SC = Autism Diagnostic Observation Schedule, communication & social interaction total score, "autism spectrum" cut-off = 7.

6.2.3.2 Group comparison: global and voxel-level

The global mean *H* was significantly lower (grey matter, GM: $t_{(61)} = 2.611$, $p = 0.011$; white matter, WM: $t_{(61)} = 2.205$, $p = 0.031$) in the ASC (GM: 0.758, SD = 0.045; WM: 0.725, SD = 0.051) than the NT group (GM: 0.788, SD = 0.047; WM: 0.755, SD = 0.057). Meanwhile, there were no significant group differences on total brain volume (NT: 1415 cm^3, ASC: 1459 cm^3, $p = 0.205$), or GM (NT: 909 cm^3, ASC: 944 cm^3, $p = 0.157$) and WM volumes (NT: 506 cm^3, ASC: 515 cm^3, $p = 0.463$). The group differences of mean *H* in GM and WM remained significant after controlling for total GM and WM volumes, respectively (GM: $p = 0.009$, WM: $p = 0.040$). In the whole-brain voxel-level analysis, four distributed clusters showed significantly lower mean *H* in the ASC group; see Figure 6-3. They involved bilateral (i) cortical midline structures: medial prefrontal cortex (MPFC, BA 10), supplementary motor area (SMA, BA 6, 8), anterior cingulate cortex (ACC, BA 32, 33), dorsal cingulate cortex (DCC, BA 24), posterior cingulate cortex (PCC, BA 23, 29, 30, 31), precuneus (PCUN, BA 7, 31) and lingual gyrus (BA 18); (ii) medial temporal structures: hippocampus, parahippocampal and fusiform gyri (HIP, PHG and FG, BA 19, 20, 35, 36, 37); (iii) lateral temporal and parietal structures: posterior superior temporal sulcus (pSTS), temporo-parietal junction (TPJ), inferior parietal lobule (IPL, BA 39, 40), superior temporal gyrus (STG, BA 22) and temporal pole (TPO, BA 38); (iv) insula and amygdala; (v) basal ganglia; (vi) thalamus; and (vii) inferior frontal gyrus (IFG, BA 44, 45).

Figure 6-3 Whole brain analysis on differences in the Hurst exponent for male adults with and without ASC

Regions identified in voxel-level whole-brain analysis all showed significantly reduced values of H in male ASC brains in distributed clusters but with specific patterns. TPJ = temporo-parietal junction; pSTS = posterior superior temporal sulcus; IPL = inferior parietal lobule; IFG = inferior frontal gyrus; STG = superior temporal gyrus; Ling = lingual gyrus; PCUN = precuneus; PCC = posterior cingulate cortex; SMA = supplementary motor area; DCC = dorsal cingulate cortex; ACC = anterior cingulate cortex; TPO = temporal pole; THA = thalamus; BG = basal ganglia; FG = fusiform gyrus; HIP = hippocampus; PHG = parahippocampal gyrus; dMPFC = dorsal medial prefrontal cortex.



6.2.3.3 Group comparison: structurally and cerebrovascularly abnormal ROIs

We then proceeded to a hypothesis-driven investigation to test group differences in *H* in regions repeatedly reported to be atypical in ASC. In these anatomical ROIs (see

Appendix Table 1), 27 of the 31 candidate atypical regions in ASC showed significantly lower *H* in ASC than in NT, with effect sizes ranging from 0.27 to 0.40 ($p < 0.032$, corrected by false-discovery-rate at $q < 0.05$). None of the control regions showed significant group differences, even at an uncorrected statistical threshold of $p = 0.05$.

6.2.3.4 Group comparison: functional social and non-social ROIs

We observed a significant group-by-ROI-set interaction ($F_{(1,61)} = 5.309$, $p = 0.025$), that the ASC participants showed significantly lower *H* than neurotypicals in the social (mean *H* for NT = 0.783, (*SD* = 0.044), ASC = 0.752, (*SD* = 0.045); $t_{(61)} = 2.751$, $p = 0.008$) but not the non-social ROIs (mean *H* for NT = 0.792, (*SD* = 0.053), ASC = 0.776, (*SD* = 0.048); $t_{(61)} = 1.219$, $p = 0.228$); see Figure 6-4 and Table 6-2.

Figure 6-4 Between-group differences in the Hurst exponent are specific to brain regions implicated in social cognitive tasks

Panel A illustrates ROIs showing consistent hypoactivation in ASC compared to neurotypical (NT) brain in social (orange) and non-social (blue) cognitive tasks. Panel B shows significantly lower mean H in the social but not the non-social ROIs in ASC (: p < .01, ns: non-significant). Error bars represent standard error of the mean. vMPFC = ventral medial prefrontal cortex; dMPFC = dorsal medial prefrontal cortex; AI = anterior insula; Amyg = amygdala; pSTS = posterior superior temporal sulcus; TPJ = temporo-parietal junction; PCC = posterior cingulate cortex; preSMA = pre-supplementary motor area; FEF = frontal eye field; IFS = inferior frontal sulcus; PMv = ventral premotor cortex; cACC = caudal anterior cingulate cortex; IPS = intraparietal sulcus; Ang = angular gyrus; VC = visual cortex.*

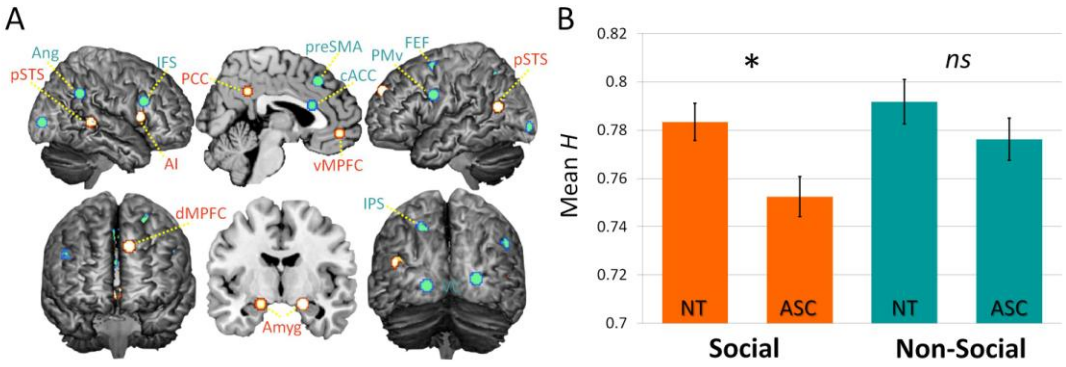


Table 6-2 Group differences of Hurst exponent (always lower in the ASC group) in social and non-social ROIs hypoactivated in ASC from meta-analyses of functional imaging studies[a]

ROIs consistently reported to be hypoactivated in social cognition task functional MRI studies (social ROIs)							**ROIs consistently reported to be hypoactivated in non-social cognition task functional MRI studies (non-social ROIs)**						
Location of ROI	Coordinate (MNI)			t	Sig.	ES	Location of ROI	Coordinate (MNI)			t	Sig.	ES
	x	y	z					x	y	z			
vMPFC, bilateral	-2	49	-7	2.34	0.023[b]	0.29	preSMA, left	2	26	44	1.70	0.093	0.21
dMPFC, left	-11	55	34	2.33	0.023[b]	0.29	FEF, left	-25	2	58	1.18	0.245	0.15
AI, right	45	14	11	-0.22	0.826	0.03	IFS, right	46	18	27	0.50	0.622	0.06
Amyg, left	-19	-8	-14	2.08	0.042[b]	0.26	PMv, left	-46	3	31	0.55	0.583	0.07
Amyg, right	20	-5	-14	2.11	0.039[b]	0.26	cACC, right	5	20	21	1.57	0.123	0.20
pSTS, right	51	-37	4	1.19	0.241	0.15	IPS, left	-22	-60	48	0.37	0.713	0.05
TPJ/pSTS, left	-45	-62	19	1.34	0.186	0.17	Ang, right	50	-50	35	1.61	0.114	0.20
PCC, bilateral	-1	-46	35	2.42	0.018[b]	0.30	VC, left	-19	-94	-2	-0.22	0.830	0.03
							VC, right	25	-90	5	0.04	0.965	0.01

[a]: Regions defined by Lombardo, et al. (2009). All statistical tests were parametric (independent sample *t* test).

[b]: $p < 0.05$

ROIs = regions of interest; MNI = Montreal Neurological Institute; t = t-statistics; Sig. = significance; ES = effect size (Pearson's *r*).

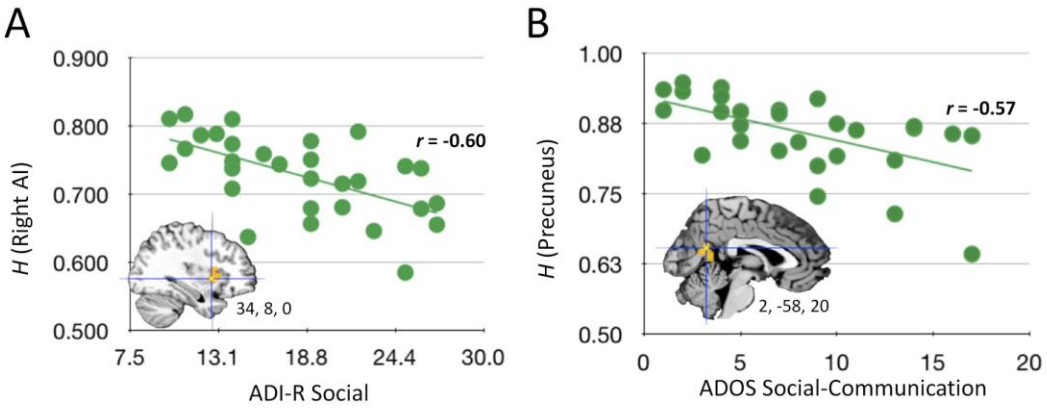
6.2.3.5 Relationship between H and autistic symptoms

H was negatively correlated with ADI-R social scores in right anterior insula (AI, peak MNI coordinate [34,8,0]; r = -0.60, adjusted R^2 = 0.26; Figure 6-5 panel A). The ADOS social-communication scores was negatively correlated with *H* in bilateral retrosplenial cortices (peak MNI coordinate [2,-58,20]; r = -0.57, adjusted R^2 = 0.21; Figure 6-5 panel B). There were no clusters showing correlation in *H* with other

symptom scores at the same statistical threshold. Mean *H* of the regions showing significant group differences (Figure 6-3) were not correlated with symptom scores.

Figure 6-5 Association between autistic symptom severity and Hurst exponent in the ASC group

Scatter plots show negative correlations of childhood ADI-R reciprocal social interaction score and current ADOS social-communication score versus H in right anterior insula (panel A) and bilateral retrosplenial cortices (in precuneus, panel B), respectively.



6.2.4 Discussion

This study tested if differences in physiological complexity of the autistic versus neurotypical brain (in males only) could be revealed by fractal analysis of resting-state fMRI time series. We confirmed that spontaneous BOLD signal fluctuations in the brain, specifically in regions implicated as atypical in previous autism neuroimaging studies, had *lower* Hurst exponents in ASC compared to typical control males,

indicating a *shift-to-randomness* of brain oscillations in the male autistic brain. Autistic symptom severity was also negatively correlated with *H* in several related regions.

6.2.4.1 Regional dynamics shifted towards randomness in ASC

Voxel-level analysis revealed lower *H* in bilateral cortical midline structures, medial temporal regions, lateral temporal-parietal and inferior frontal areas, and subcortical structures in ASC. These regions overlapped largely with the structurally implicated ROIs in a complementary hypothesis-driven analysis. At a functional level, lower *H* was also observed in male adults with ASC across regions that are consistently hypoactive during social tasks, but not for regions hypoactive during non-social tasks. Most importantly, there were no group differences in areas where abnormalities had not been consistently reported, such as the primary sensory-motor and visual cortices. Thus, fractal analysis was effective in detecting atypical neural organisation in regions previously reported to be atypical in autism, characterised by a shift of brain dynamics to randomness compared to the neurotypical brain. A shift-to-randomness in *whole-brain network organisation* has recently been shown using graph theory approach in schizophrenia (Rubinov et al., 2009) and Alzheimer's disease (Stam et al., 2009). Our findings have further demonstrated that changes in randomness of measurable neurophysiological signals are also seen in ASC, at the level of *regional organisation*.

The regions appear to fall into three categories: *social brain* (Adolphs, 2009; Blakemore, 2008; C. D. Frith, 2007), *connection hubs*, and regions related to *motor planning and coordination*. Cortical midline structures (e.g. MPFC and cingulate) and the hippocampal formation form a neural circuit involved in self-projective/simulation processes (Buckner & Carroll, 2007) such as mentalizing, self-referential cognitive

processing (Amodio & Frith, 2006; Lombardo, Chakrabarti, Bullmore, Wheelwright, et al., 2010), autobiographical memory and prospection into the future (Schacter, Addis, & Buckner, 2008), perspective taking (Ruby & Decety, 2001), scene construction and spatial navigation (Hassabis & Maguire, 2007). Together with IPL they constitute the 'default network' (Buckner, Andrews-Hanna, & Schacter, 2008). Several studies implicate atypical dMPFC recruitment in ASC for self-referential emotion (Silani et al., 2008) or mentalizing (Castelli, Frith, Happe, & Frith, 2002; Kana, Keller, Cherkassky, Minshew, & Adam Just, 2008; Wang, Lee, Sigman, & Dapretto, 2007), and atypical vMPFC activity during self-representation in this sample (Lombardo, Chakrabarti, Bullmore, Sadek, et al., 2010). Relatively lower functional connectivity among these regions has been repeatedly observed in ASC (Kennedy & Courchesne, 2008; Monk, et al., 2009; Weng, et al., 2010). Lateral temporal regions, including pSTS/TPJ, STG and temporal pole, also serve as core components in the social brain and are integral for understanding movements, perspective-taking and convergence of social knowledge (C. D. Frith, 2007). Inferior frontal areas (BA44), integral for language function and the mirror system (Iacoboni & Dapretto, 2006), are also atypical in ASC (Oberman & Ramachandran, 2007). The insula and amygdala are critical for bottom-up automatic affective (Adolphs, 2009; Ochsner, et al., 2009; Wager, et al., 2008) and evaluative processing (Cunningham, et al., 2004), empathy (Singer, et al., 2004; Wicker, et al., 2003) and interoceptive awareness (A. D. Craig, 2002; Critchley, et al., 2004). Previous fMRI studies in ASC highlight hypoactive responses during face-processing and emotion tasks in the insula and amygdala (Lombardo, et al., 2009).

In summary, we suggest that the specificity with which more random brain dynamics affect these social brain components, whereas other neural circuits crucial for

non-social cognitive processes are relatively unaffected, is not a coincidence. Social cognition likely relies on highly dynamic and coordinated information processing (Minshew, et al., 2006) and our observations of *shift-to-randomness* may be a critical physiological indicator for atypical social cognition in ASC. Certainly some of these regions already show evidence of association with atypical social-communication function, in particular the right anterior insula and retrosplenial cortex (see Figure 6-5); this right insula locus (34, 8, 0) is also close to the right insula region (34, 0, -2) showing significant correlation of ACC-insula functional connectivity to social competence in healthy volunteers (Di Martino et al., 2009). However, these two areas are only part of a distributed set of regions showing more random signals in the autistic than the neurotypical brain in males, and average *H* in those other regions is not correlated with symptom scores. This indicates that signal randomness is correlated with symptoms only in very specific regions and that *H* should not be treated as a direct reflection of social-communication function.

Connection hubs have dense connections to the rest of the brain. PCC has been identified as the structural core of the cerebral cortex (Hagmann et al., 2008) and is also a major functional connector hub (Buckner et al., 2009) associated with the default network (Buckner, et al., 2008). The thalamus is a sensory relay station characterised by extensive thalamocortical interconnections and the reciprocal nature of these neuronal loops (Behrens et al., 2003; Llinas, Ribary, Contreras, & Pedroarena, 1998). In the autistic brain the thalamus has been shown atypical in its hyperconnectivity to the cerebral cortex (Mizuno, Villalobos, Davies, Dahl, & Muller, 2006), altered neurochemical composition (Hardan, et al., 2008) and relative size to total brain volume (Hardan, et al., 2006), and lower relative glucose metabolic rates (Haznedar, et

al., 2006). The more random signal in these regions in ASC may reflect perturbed information organisation.

Finally, regions crucial for motor planning and coordination (i.e., SMA, basal ganglia and possibly parts of cerebellum) also possessed more random signals in ASC. This may be relevant to the motor dysfunction in autism (Nayate, Bradshaw, & Rinehart, 2005). However, more research is needed to assess whether this shift towards randomness has a bearing on network organisation or atypical processing associated with these regions.

6.2.4.2 Physiological meaning of H

The physiological meaning of *H* is obscured by our limited understanding at present on the relative contributions of neuronally- and blood-supply mediated sources to the measured BOLD signal (Leopold, 2009), as well as cardiac and respiratory signal confounds, and indeed the global signal. Nevertheless, we are willing to speculate that fractal scaling may serve as an indicator of the organisation and coordination properties of the local neural circuits, although we are not able to delineate the relative contribution of each at this stage. The *shift-to-randomness* may imply less coordinated signal organisation at the local level of possibly small-scale neural circuits. Preliminary analyses indicated that differences between pair-wise regional *H* were negatively correlated with their BOLD signal low-frequency correlation coefficients (see Appendix Figure 1), suggesting a possible but complicated link between fractal scaling and functional connectivity. That is, the more similar fractal scaling of the BOLD signals of a pair of regions (indicated by smaller difference in *H*), the higher their functional connectivity. Future correlation studies of fMRI fractal parameters with other regional measurements (e.g. grey matter density, local electromagnetic signal

property, regional blood flow or chemical composition) as well as functional connectivity may help illuminate their exact physiological significance.

The aging-related decrease of fractal dimension (*increased H*) depicted in previous studies (Maxim, et al., 2005; Wink, et al., 2006) corresponds to the fractal theory of aging (Goldberger et al., 2002), in which hippocampal dynamics become less complex and more predictable. It is thus plausible that the lower *H* observed in the ASC relative to the NT brains reflects a reduction in aging-related decline in biological processes of the brain given that recent studies showing brain maturation in adults with ASC might be atypical and delayed (McAlonan, et al., 2002; Raznahan, Toro, et al., 2010). We were not able to test this formally in the present study given the insufficient sample size and relatively narrow age-range of the participants. The physiological range of *H* for different brain structures, as well as their trajectories of change in the course of brain maturation awaits further investigation. This may establish a clinical application for the Hurst exponent not currently available due to incomplete knowledge of the physiological processes that underpin its value, normal range, and sensitivity and specificity to different neuropsychiatric conditions.

6.2.4.3 Implications for the neurobiology of autism

Our findings have potential implications for theories of atypical connectivity (Baron-Cohen & Belmonte, 2005; Belmonte, et al., 2004) in ASC. The hypothesis of local (small-scale circuit) over-connectivity (Baron-Cohen & Belmonte, 2005; Belmonte, et al., 2004; Rubenstein & Merzenich, 2003) has so far been supported by evidences of early brain overgrowth (Courchesne, et al., 2007), cerebral cortical minicolumnopathy (Casanova, et al., 2006) and localised enlargement of cerebral radiate white matter (Herbert et al., 2004). These perspectives argue that local structural

abnormalities and disorganisation result in local over-connectivity at the cost of decreased long-range information processing. The *shift-to-randomness* of BOLD signals in the implicated regions in the autistic brain may represent perturbed information processing within small-scale circuitry that may in turn have more profound effects on large-scale circuitry.

6.2.4.4 Limitations

Several caveats should be mentioned. First, inferences from fractal analysis are constrained by insufficient current knowledge of its exact physiological relation. This will become clearer with further research tying fractal parameters to other measurements. Second, since in some participants the most inferior part of cerebellum was not fully covered in the images, we were not able to make confident inferences about this important structure in autism neurobiology (Amaral, et al., 2008). Finally, since this study was limited to high-functioning male adults, it is unknown how the results may generalise to females and individuals of other ages or intellectual levels. The test for female population will be reported later in Study 2 of this chapter. We are also agnostic about whether the observed changes in fractal dynamics are specific to ASC. Further clarity should become available when similar studies are conducted on other neuropsychiatric, especially neurodevelopmental conditions, such as intellectual disability, schizophrenia or attention deficit/hyperactivity disorder.

6.3 Study 2: Differential Complexity of Resting Brain Oscillations between Sexes and in ASC

6.3.1 Introduction

Study 1, as a proof of method, has demonstrated that for males, fractal analysis on the complexity of brain oscillations in resting-state fMRI is capable of capturing a *shift-to-randomness* in regions previously reported to be atypical in ASC (Lai et al., 2010). Whole-brain analysis is as sensitive as the hypothesis-driven ROI approach in identifying regional group differences. Study 2 built on this novel but sensitive methodology, further explored if females with and without ASC also showed distinct brain oscillations in GM, and if there was an interaction between sex and ASC at the level of functional organisation, as shown in Chapter 5 on structural morphometry.

6.3.2 Methods

6.3.2.1 Participants

Female participants with and without ASC were recruited through the same procedures as for males, described in detail in earlier chapters, from November 2009 to October 2010. Thirty-four typical female adults completed fMRI scanning and three were excluded due to image acquisition problems (incomplete brain coverage), leaving 31 available participant images. Thirty-three women with ASC fulfilled inclusion criteria in terms of ASC presentation and completed fMRI scanning; three were excluded (two with incomplete brain coverage and one with marked motion artefact), leaving 30 available participant images. To match for age and IQ across all

four groups, the final cohort comprised of 33 typical male adults, 29 male adults with ASC, 30 typical female adults, and 30 female adults with ASC; see Table 6-3 for details.

6.3.2.2 Resting-state fMRI data acquisition, pre-processing and time series modelling

Image data acquisition, pre-processing and estimation of *H* were performed exactly the same way as described in Study 1.

6.3.2.3 Statistical analysis

The same statistical analysis strategy was applied as in Study 1. For Study 2 we performed only whole-brain voxel-level analysis. For planned pair-wise independent sample whole-brain analysis (i.e., between women with and without ASC, and between typical men and women; see results for detail), GLMs were regressed onto voxel estimates of *H*, with group as the independent variable. For a summary two-way factorial design analysis, sex and diagnosis were the independent variables. The standardised regression parameter for the independent variable was tested for statistical significance by non-parametric procedures. We used a permutation test which adjusted the probability threshold for statistical significance of multiple comparisons by controlling the expected number of false positive cluster-level tests to be less than one per map (Bullmore, et al., 1999; Suckling & Bullmore, 2004; Suckling, et al., 2006).

For correlations with measures of autistic symptoms for women with ASC, GLMs were regressed to voxel estimates of *H* with autistic symptoms as the independent variable and global mean *H* as a covariate. For a final correlation analysis on males and females with ASC altogether, to identify regions showing interaction between sex and

autistic symptom measures previously found correlated with *H*, a GLM was regressed to voxel estimates of *H* with sex and symptom measure (i.e., ADI-R social interaction or ADOS social communication domain scores) as the independent variables.

6.3.3 Results

6.3.3.1 Demographics and behavioural characteristics

All groups (MC: typical control male adults; MA: male adults with ASC; FC: typical control female adults; FA: female adults with ASC) were pair-wise matched on age and full-scale IQ; for subscales FC scored higher than MC on verbal IQ and MC scored higher than FA on performance IQ, under a non-corrected threshold of $p < 0.05$. For autistic traits, the pattern of group difference was the same as illustrated in Chapter 3. For the ADI-R, MA scored slightly higher than FA in the RSB domain (under a non-corrected threshold of $p < 0.05$) but they had the same severity on the reciprocal social interaction and communication domains. As reported in Chapter 2, MA scored significantly higher than FA in the ADOS scores even after multiple comparison correction.

Table 6-3 Demographic and behavioural characteristics for the four groups

	MC (N=33) Mean (SD)	MA (N=29) Mean (SD) [range] [$]	FC (N=30) Mean (SD)	FA (N=30) Mean (SD) [range] [$]	Statistics [&]
Age (Years)	28.4 (6.1)	26.9 (7.5)	27.6 (6.5)	28.3 (8.4)	ns
Verbal IQ	110.8 (12.0)	112.6 (13.8)	118.9 (10.3)	114.1 (15.7)	FC>MC (p = .015)
Performance IQ *	118.5 (11.4)	112.4 (15.1)	116.2 (8.9)	110.3 (17.4)	MC>FA (p = .018)
Full IQ *	116.3 (11.6)	113.9 (14.5)	120.0 (8.2)	113.9 (15.8)	ns
AQ	15.2 (6.9)	33.2 (7.8)	11.6 (4.8)	38.3 (6.5)	◎
ADI-R [#]					
Social	–	17.8 (5.3) [10 – 27]	–	17.0 (5.0) [11 – 29]	ns
Communication	–	15.3 (3.6) [8 – 22]	–	13.6 (4.6) [8 – 25]	ns
RSB	–	5.8 (2.5) [2 – 10]	–	4.4 (2.0) [2 – 10]	MA>FA (p = .034)
ADOS [@]					
S + C	–	8.5 (4.9) [1 – 17]	–	4.8 (4.5) [0 – 19]	MA>FA (p = .002)
RSB	–	1.1 (1.0) [0 – 4]	–	0.1 (0.3) [0 – 1]	MA>FA (p < .001)

[$]: For ADI-R and ADOS scores.

[&]: Independent sample *t*-tests. All *p* values were **not** corrected for multiple comparisons.

*: Levene's Test for Equality of Variances showed significant non-equal variances, therefore equal variance was not assumed in the statistical tests.

[#]: N = 29 for MA, N = 27 for FA.

[@]: Distribution of scores significantly deviant from normal, therefore non-parametric Mann-Whitney tests were performed for group comparison of ADOS algorithm scores.

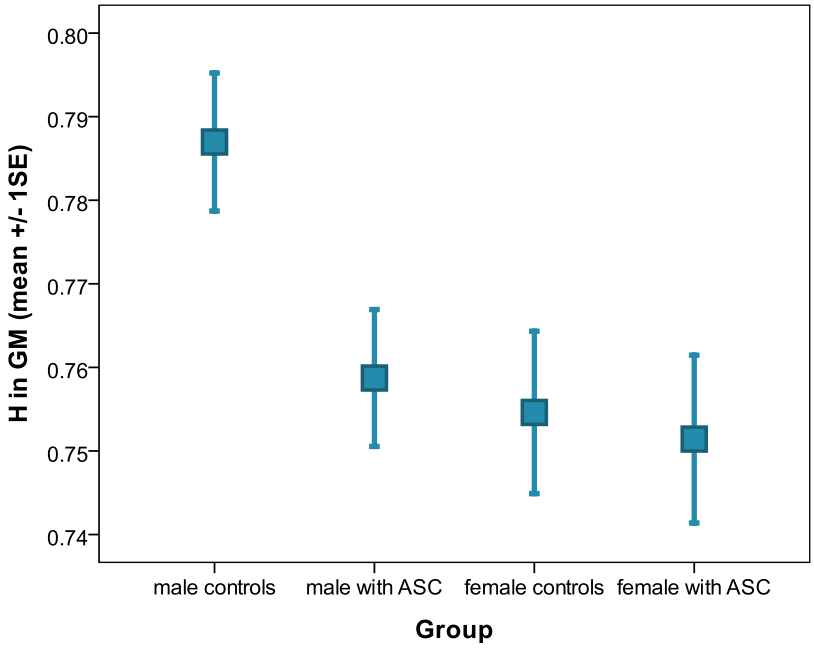
MC = (neuro)typical control group male adults; MA = male adults with ASC; FC = (neuro)typical control group female adults; FA = female adults with ASC; SD = standard deviation; ns = non-significant ($p > 0.05$); ◎ = the same statistical pattern amongst groups as described in Chapter 3, see the result section of Chapter 3 for detail; AQ = Autism Spectrum Quotient; ADI-R = Autism Diagnostic Interview-Revised; RSB: repetitive, restrictive and stereotyped behaviour; ADOS: Autism Diagnostic Observation Schedule; S + C: ADOS "social interaction + communication" total scores.

6.3.3.2 Group comparisons: global level

A two-way factorial ANOVA with global mean *H* in GM as the dependent variable, and sex and diagnosis as fixed factors, showed a significant main effect of sex ($F_{(1,118)} = 4.77$, $p = 0.031$), a marginal main effect of diagnosis ($F_{(1,118)} = 2.994$, $p = 0.086$), but a non-significant interaction ($F_{(1,118)} = 1.902$, $p = 0.17$). Overall males had higher *H* than females. Planned pair-wise comparisons confirmed that MC had higher *H* than MA (MC: 0.787, SD = 0.047; MA: 0.759, SD = 0.044; $t_{(60)} = 2.417$, $p = 0.019$) as already shown in Study 1, but FC was not different from FA (FC: 0.755, SD = 0.053; FA: 0.751, SD = 0.055; $t_{(58)} = 0.228$, $p = 0.82$). This, however, did not reach a significant sex-by-ASC interaction. Contrasting the typical control groups demonstrated a typical sex difference in global mean *H* ($t_{(61)} = 2.552$, $p = 0.013$). See Figure 6-6 for illustration.

Figure 6-6 Global mean H in GM across four groups

There was a typical sex difference in global mean H in GM. Men with ASC had a lower global mean H than those without, but this diagnostic group difference did not occur in females.



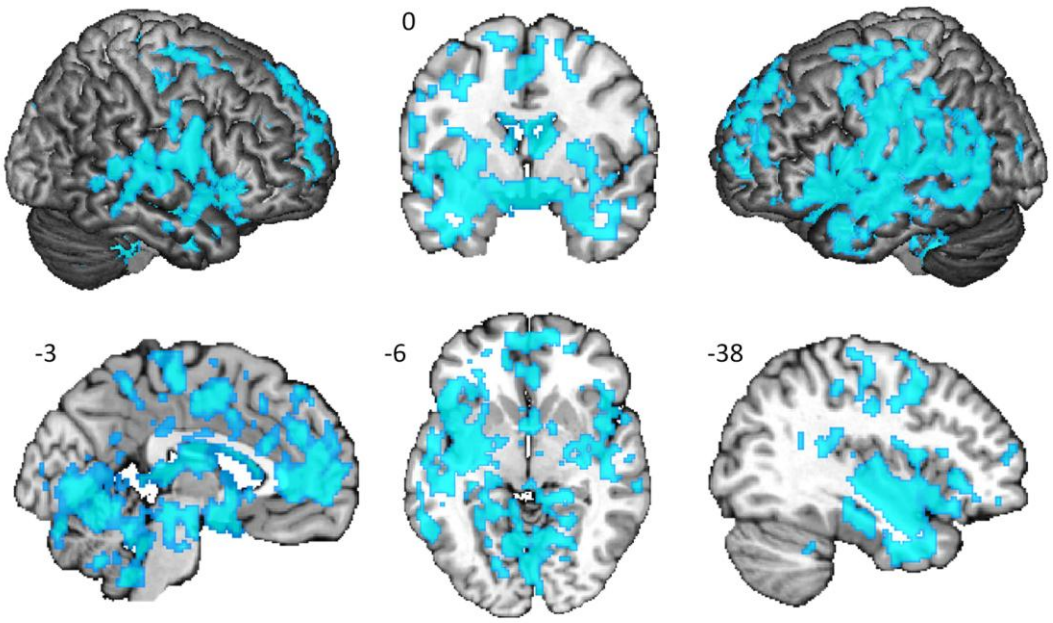
6.3.3.3 Group comparisons: voxel level

We first tested, for women with and without ASC, if the voxel-level comparison of *H* would show group difference in brain regions reported to be atypical in ASC as revealed in Study 1. Surprisingly, no region showed significant group difference. A two-way factorial design GLM subsequently showed four clusters of regions with significant sex-by-ASC interaction (1 error cluster per image, equivalent $p = 0.0025$, two-tailed). These regions all fell into those showing diagnostic group difference in males in Study 1 (see Figure 6-3), and the pattern was always MC > MA but FC = FA. A post-hoc comparison between typical men and women showed one extensive

cortico-subcortical cluster with lower *H* in women, sparing mainly the lateral aspects of the parietal and occipital lobes and part of frontal lobes (1 error cluster per image, equivalent $p = 0.0026$, two-tailed); see Figure 6-7.

Figure 6-7 Typical sex difference in H in the brain

Regions in light-blue show significantly lower H in typical women compared to typical men. A range of cortical and subcortical structures were involved, sparing mainly the lateral aspects of the bilateral parietal and occipital cortices and part of frontal lobes.



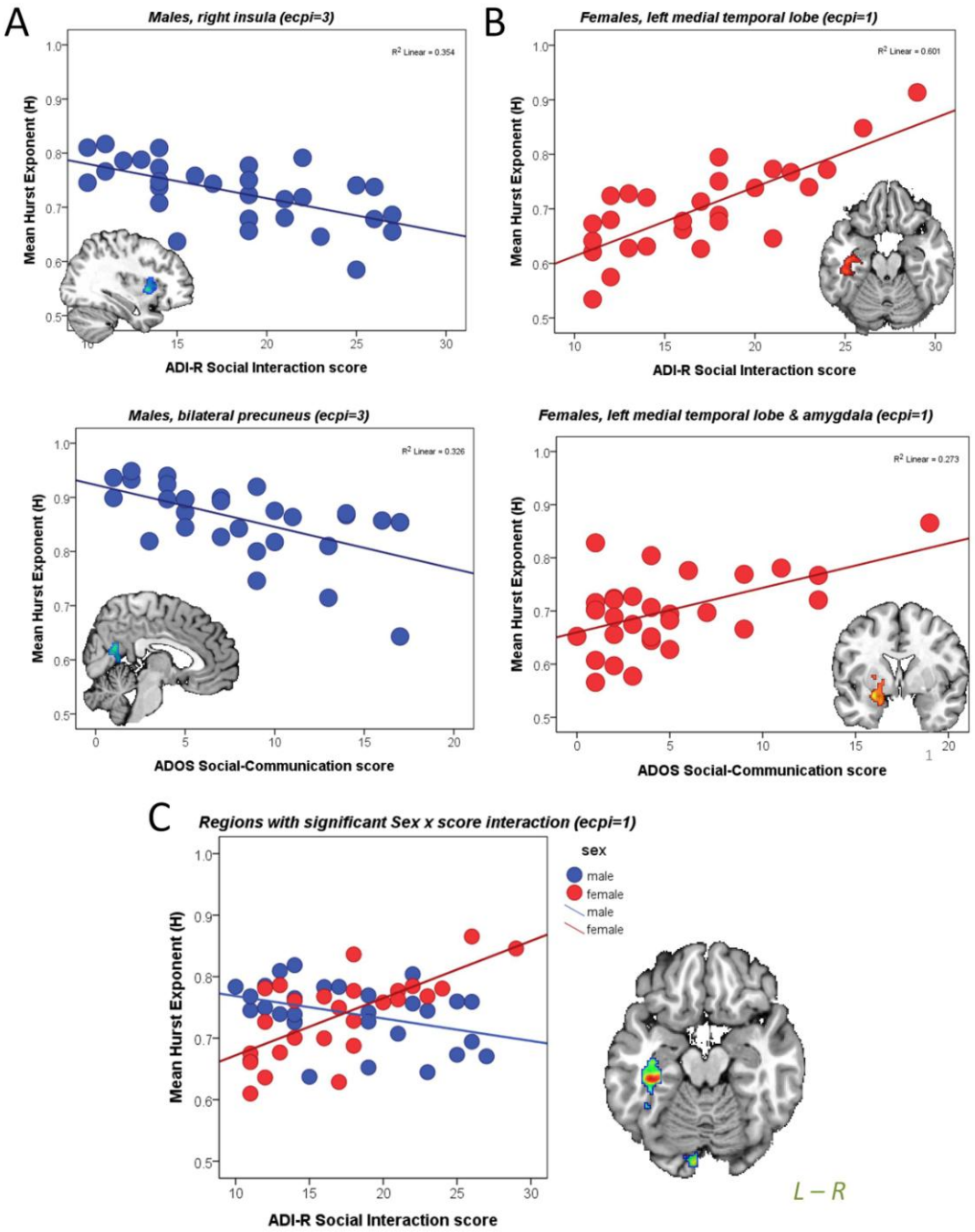
6.3.3.4 Correlation with autistic symptom measures

In contrast to the negative correlation of *H* to symptom measures (ADI-R social interaction and ADOS social-communication domain scores) in male adults with ASC identified under a relaxed threshold (3 error clusters per image, equivalent $p = 0.0059$,

two-tailed; see Figure 6-8 panel A) in Study 1, female adults with ASC showed a *positive correlation* of *H* with these measures in left medial temporal lobe under a stringent threshold (1 error cluster per image, equivalent $p = 0.0018$, two-tailed; see Figure 6-8 panel B. Upper plot for ADI-R social interaction score: left fusiform, hippocampal and parahippocampal gyri, peak MNI coordinate [-42,-28,-16], $r = 0.78$; lower plot for ADOS social-communication score: left amygdala, ventral putamen, fusiform, hippocampal and parahippocampal gyri, peak MNI coordinate [-32,-14,-30], $r = 0.52$). For ADI-R social interaction score, the region also showed a significant sex-by-score interaction in a confirmatory GLM on all ASC participants (1 error cluster per image, equivalent $p = 0.0025$, two-tailed; see Figure 6-8 panel C; male: $r = -0.40$, female: $r = 0.70$), substantiating the sex difference in correlation pattern. No correlation was found for other symptom scores in women with ASC.

Figure 6-8 Correlation of H with autistic symptoms in men and women with ASC

Panel A duplicates Figure 6-5 to illustrate the negative correlations of H to ADI-R social interaction domain score at right insula (upper) and to ADOS social-communication domain score at precuneus (lower) in men with ASC. The pattern is in sharp contrast to panel B, which illustrates in women with ASC the positive correlation of H to ADI-R social interaction domain score at left medial temporal lobe including fusiform, hippocampal and parahippocampal gyri (upper) and to ADOS social-communication domain score at left amygdala, ventral putamen and medial temporal lobe, again including fusiform, hippocampal and parahippocampal gyri but slightly anterior (lower). Panel C shows a significant 'sex-by-ADI-R social interaction score' interaction at left medial temporal lobe, which confirmed the sex difference in correlation pattern at this region. ecpi = error cluster per image.



6.3.4 Discussion

Study 2 extended the tests from males to females on characterising the physiological complexity of brain oscillations using fractal analysis on resting-state

fMRI time series, and revealed two findings of major interests. For the first time we showed a typical sex difference in the complexity of resting brain oscillations. Second, we found the effect of ASC on brain oscillations dependent on sex.

6.3.4.1 Typical sex differences in brain oscillations

Sex differences in resting-state brain activity have rarely been investigated. The only study to date demonstrates that females have stronger connectivity within the default network (by both seed-based and independent component analyses), and locally stronger power of low-frequency oscillations at PCC (Biswal, et al., 2010). However the physiological meanings of these subsystem- and region-specific sex differences are still unknown. Up to now there has been no report of typical sex differences in other characteristics of resting brain activity, nor reports from previous fractal analyses on resting brain oscillations (Maxim, et al., 2005; Wink, et al., 2006; Wink, et al., 2008). We demonstrated here for the first time a higher randomness in brain oscillations in typical female compared to male adults, in diffuse cortical and subcortical structures apart from the lateral parietal, occipital and part of frontal cortices. How this is explained physiologically in terms of its emergence (e.g. hormonal or genetic effects on brain function) or cognitive correlates (e.g. sex differences in resting mental activity) awaits further investigation. At this stage, the main messages are that the typical physiological range of *H* may be sexually dimorphic, and that future investigations on resting brain activity should be explored with sex stratified.

6.3.4.2 Sex-specific effect of ASC on brain oscillations

The marked interaction between sex and ASC in *H* echoes the above suggestions.

For the three models (OG, SSE and EMB, see Chapters 1 and 5 for detail) relating sex and ASC in brain oscillation pattern (which reflects functional organisation), OG is rejected due to the evident interaction effect noted in the two-way factorial design GLM voxel-level analysis, and the EMB pattern is not present at all due to the absence of an EMB *directionality* (i.e., the direction of difference in males with and without ASC does not fit with the direction of typical sex difference). Therefore SSE is the best fit here. More importantly, regions with significant interaction all showed the pattern of *a shift-to-randomness in males but not in females*, suggesting that brain oscillation complexity change is characteristic only for male but not for female adults with ASC. SSE is further supported by the sex-by-score (ADI-R social interaction) interaction on *H* in medial temporal structures, where the correlations between *H* and symptom score were positive in females but negative in males. Future studies on ASC using resting-state fMRI should carefully investigate if SSE patterns exist. If SSE is repeatedly found in brain functional organisation, it will evidence sex-specific neuro-functional phenotypes and suggest sex-specific contributing mechanisms.

The SSE pattern noted here in brain oscillations is also in marked contrast to the SSE pattern found at the structural level. SSE in brain morphometry is actually an SSEMB pattern, which is different from the pure SSE noted here. Furthermore, for brain morphometry the diagnostic group difference is more evident in females than in males, whereas for brain oscillations it is just the opposite. This suggests multi-level complexity of sex-ASC relationship in neurobiology, which will be discussed integrally in Chapter 7.

6.3.4.3 Correlation with symptom measures

A voxel-level mass-univariate approach did not identify specific regions with

different oscillatory complexity between women with and without ASC, but within those with ASC, *H* in medial temporal lobe and amygdala positively correlated with both childhood and current socio-communication symptom severity. This indicates that the more socio-communication impairments, the *simpler* (more *regular*) the intrinsic oscillations in these structures. Providing the critical roles of these regions in memory (D. L. Clark, et al., 2010), automatic affective processing (Adolphs, 2009) and the involvements in the default network (Buckner, et al., 2008), that all have been reported to be atypical in ASC, it is interesting to observe that *H* picked up these structures in relation to the socio-communication symptoms in women with ASC. However, the sex-by-score interaction of *H* in medial temporal lobe points out the sex-dependent nature in correlation patterns, indicating how *H* is relevant to the socio-communication features in ASC is determined by sex. This again emphasises the importance of stratification by sex for future investigations for ASC. However, as stated in Study 1, we refrained ourselves from further inferences on how *H* reflects autistic symptoms owing to the insufficient knowledge of its exact physiological underpinnings at this stage.

6.3.4.4 Limitations and future directions

All the limitations discussed in Study 1 apply to Study 2. Furthermore, since this is the first study demonstrating sexual dimorphism in brain oscillations, it requires independent replication. A potential strategy is to apply the methodology to publicly available large datasets (e.g. The 1000 Functional Connectomes Project, http://www.nitrc.org/projects/fcon_1000/). It is also crucial to investigate how *H* relates to other region-specific measures for resting-state fMRI, in order to provide further insights into its physiological implications. Basic neurobiological works, for

example investigating the oscillation patterns at the neuronal and the neural circuitry levels, may provide the most progress in elucidating the physiological underpinnings of the fractal geometry of intrinsic brain oscillations. Lastly, future attention should also focus on the inter-modality relationship in neuroimaging (e.g. how *H* is related to structural measures), and how they conjointly help illustrate the neurobiological phenotypes of ASC.

Chapter 7

General Discussion

7.1 Summary of Major Findings

This dissertation has investigated how the characteristics of ASC in high-functioning adults vary by sex. Relationship between sex and ASC was tested using three models derived from both statistical principle and cognitive theories of autism, namely the orthogonal (OG), sex-specific effect (SSE) and the extreme male brain (EMB) models. Levels of investigation include behaviour, cognition, structural and functional aspects of the brain. This dissertation provides initial steps to characterise females with ASC and to understand how they are similar to and different from males with ASC as compared to respective typical counterparts. It also serves as the basis to generate hypotheses for future investigations regarding the underlying mechanisms for the emergence and development of ASC.

7.1.1 Behaviour and cognition

In terms of behaviour and cognition male and female adults with ASC are neither the same, nor are they totally different. We compared male and female adults with clinically diagnosed ASC and above-threshold (in terms of ADI-R cut-offs) childhood symptoms, without intellectual disability or major comorbidities. They were similar in the severity of childhood autistic presentation, current dispositional traits related to autism, co-occurring psychiatric symptoms, poorer than normal social cognitive abilities (mentalizing and basic emotion recognition) and signal detection sensitivity. They were however different in current interpersonal interaction (females had markedly less recognisable autistic characteristics), lifetime sensory issues (more in females), self-perceived autistic traits (females reported more traits), relation between

developmental language delay and current IQ (history of language delay correlated with current IQ in females only), perceptual attention to detail and motor executive function (males with ASC performed poorer than typical males, but females with ASC performed similarly as typical females).

The statistical relationship between sex and ASC is different by the different domains of behaviour and cognition. When it is about the defining features of ASC, e.g. the childhood symptoms, dispositional traits, mentalizing and emotion recognition abilities, it usually conforms to the OG model which indicates that ASC influences males and females equally. For dispositional traits there is a good fit with the EMB model, where a typical sex difference is attenuated due to the influence of ASC. On the other hand, when it comes to the less central features of ASC, e.g. perceptual attention to detail or motor executive function, the SSE model best describes the sex-ASC relationship by indicating that ASC has a different effect on males versus females – it impairs males but relatively spares females. The potential implication of this *central-peripheral discrepancy of sex-ASC relationship* will be discussed later.

7.1.2 Structural aspect of the brain: morphometry

The neuroanatomical features for female adults with ASC compared to typical female adults were first identified by voxel-based morphometry (VBM). Women with ASC had smaller relative grey matter (GM) volume in the bilateral anterior and middle cingulate cortices, supplementary motor areas, insula, right temporal pole, calcarine and superior cerebellum. For white matter (WM) they had larger posterior brain regions bilaterally involving the arcuate fasciculus, internal capsule, corpus

callosum, cingulum and inferior longitudinal fasciculus. They also had smaller bilateral ponto-cerebellar fibres and the right corticopontine fibres. This pattern, however, is very different from the consensus morphometric features for males with ASC compared to typical males, that in GM they have smaller bilateral amygdala-hippocampal complex and precuneus, and have a larger region at left middle inferior frontal gyrus (Via, et al., 2011). In WM males with ASC, compared to typical males, show larger anterior portion of the right arcuate fasciculus, anterior portion of the left inferior fronto-occipital fasciculus and the superior portion of the left uncinate fasciculus (Radua, et al., 2011). This dissimilarity between sexes suggests that males and females with ASC differ in terms of their characteristic brain morphometric features.

Further comparisons across males and females, with and without ASC support this suggestion. In a two-way factorial design, we found that in terms of spatial characteristics, the SSE model best described sex-ASC relationship in WM by showing regions with significant sex-by-ASC interaction (i.e., *SSE regions*). This suggests a sex-dependent ASC effect in brain morphometry. In GM the interaction was however marginal. The next step to test if the EMB model also explained morphometric differences by spatial overlap analyses further demonstrated that the typical masculinisation pattern overlapped substantially with the ASC-control group difference pattern *in females but not in males*, for both GM and WM. In WM the overlap regions (i.e., *EMB regions*) located exactly at the SSE regions discovered earlier. This confirms that there is a significant sex difference in the extent a masculinisation pattern corresponds to the effect of ASC on brain morphometry. Therefore, the best model to explain the sex-ASC relationship on brain morphometry

is a *sex-specific extreme-male-brain pattern* (i.e., SSEMB model). This effect was weaker in the GM due to the trend significance of SSE.

There was also initial evidence showing correlations of SSEMB region size to the proxy index of prenatal androgen exposure effect (i.e., 2D:4D ratio) in typical females but not in females with ASC. Together with the lack of an EMB pattern in the male groups, these findings may be explained by *ceiling effects* in volumetric masculinisation but other modulating factors may also contribute. This provides one (out of many) plausible mechanistic level future research direction.

7.1.3 Functional aspect of the brain: intrinsic oscillations

The brain functional aspect was investigated by the BOLD signal complexity of intrinsic brain oscillations during resting-state. A fractal parameter, the *Hurst exponent* (*H*), was able to identify regions previously reported atypical and characterising autism in male adults with ASC. This parsimonious parameter is able to describe resting-state brain oscillations in a region-specific manner.

We then, for the first time, discovered a typical sex difference on the complexity of intrinsic brain oscillations, indicated by *H*, over broad brain GM regions sparing mainly the lateral parietal, occipital and parts of frontal lobes. Furthermore, in contrast to what was found for males, females with or without ASC showed similar patterns of brain oscillations, both globally and locally. Therefore, the SSE model again best describes the differences among groups in terms of intrinsic brain activities. However this SSE pattern is different from the structural one. For brain morphometry ASC effect was evident in females (with an EMB pattern) yet much less in males, but for intrinsic oscillations it was the opposite and no EMB pattern was identified.

7.2 Implications

By integrating the observational findings across behavioural, cognitive and neurobiological levels, several implications are revealed in relation to the recognition of similarities and differences between males and females with ASC, the explanation to why sex-ASC relationship is not consistent across levels, the directions for future mechanistic investigations and the practical implications for research and clinical services.

7.2.1 Males and females with ASC are both similar and dissimilar

The first aim of this dissertation is to characterise female adults with ASC, who are under-studied, potentially under-recognised and mis-diagnosed (Attwood, 2007; Attwood & Grandin, 2006; Baron-Cohen, et al., 2011; Kopp & Gillberg, 2011; Miller, 2003; Wing, et al., 2011). The characterisation outlined in the previous chapters provides the initial steps to facilitate a better understanding and recognition of females with ASC, and may inform the revision of the diagnostic criteria (Wing, et al., 2011).

Some of the similarities and differences between males and females with ASC can be described by direct comparison between the two groups (e.g. childhood autistic symptoms). However for aspects with a presence of typical sexual dimorphism (e.g. brain morphometry), the description is sensible only when comparing how males and females with ASC are respectively different from their same-sex typical counterparts. A direct comparison between males and females with ASC in this instance will be inevitably confounded by the presence of typical sex differences. A presence of SSE instead properly indicates sex differences within ASC for these aspects.

We noted that adult males and females with ASC are *both similar and dissimilar*. They are similar in the defining core behavioural and cognitive features of ASC. This is not surprising since the way we selected our sample was to conservatively follow the principle in solving the *soft* side (see Chapter 1) of the behavioural level problem, i.e., the included participants all had severe enough, and perhaps also 'typical enough' core autistic features (in their childhood). Therefore, the inclusion criteria may already set the theme to find sex similarities in the core domains, including childhood autistic symptoms, current dispositional traits related to autism, co-occurring psychiatric symptoms and social cognitive abilities.

What is most interesting is the dissimilar part. For the less central cognitive features, and both the structural and functional aspects of the brain, SSE is the best descriptive model. This suggests that *males and females with ASC are also dissimilar* – perhaps the most important message from this series of studies. We interpreted the SSE in the 'peripheral' behavioural and cognitive domains as reflecting the clinical heterogeneity of ASC with regards to sex. The brain findings further suggest that this sex-related heterogeneity lies in a more fundamental level. That is, although both sexes show similar core defining features of ASC, their brain characteristics are different. The contrast between sex-similarity in the core ASC behavioural and cognitive presentations and sex-difference in the brain may indicate common phenotype but different endophenotype, or even different underlying genetic liability and/or environmental contributors for each sex (McCarthy & Arnold, 2011). This raises the possibility of *sex-differential causal models* for ASC (Morton, 2004) which needs to be borne in mind in designing future research on the endophenotype, developmental mechanism and aetiology of ASC. Falsification requires research

designs capable of demystifying the puzzles at the mechanism level.

Not all cognitive features relevant to ASC or sex were examined in the present studies, neither were other aspects of brain structure (e.g. geometric features) or function (e.g. functional connectivity). Therefore, it is not certain to what extent the SSE model accurately describes the wide array of characteristics in ASC. Future observational investigations will provide illumination. The ultimate goal, however, is to elucidate causality and mechanism by explaining why males and females with ASC are similar to and different from each other.

The observations here have implications to the broader field of psychiatry and developmental psychopathology as well. Sex differences within neuropsychiatric conditions are rarely emphasised, for reasons described in Chapter 1 that this relates to the *hard* side of the problem. For disorders where the diagnosis relies on behavioural criteria, by definition sex differences can only be observed in aspects other than the core defining features. However, those sex differences may not be at the centre of concerns (e.g. peripheral cognitive features) or may be hard to notice (e.g. brain features). This is what we have observed for ASC. Sex-specific characteristics in the brain (and the potential underlying mechanisms) may be masked by a common core behavioural/cognitive phenotype, an example of *equifinality* in developmental psychopathology (Cicchetti, 2006). If *equifinality related to sex* is common, it could act as a basic principle underlying the heterogeneity of neuropsychiatric conditions, including ASC. To clarify whether it occurs similarly or differentially in male-predominant (e.g. neurodevelopmental conditions), female-predominant (e.g. affective disorders) or sex-balanced (e.g. psychotic disorders) neuropsychiatric conditions will further disentangle how sex affects the emergence of these conditions.

7.2.2 Sex-ASC relationship in the brain varies by structural versus functional aspects

Another aim of this dissertation is to elucidate how sex relates to ASC at different levels of investigation. As described earlier, apart from the core cognitive behavioural features, the SSE model dominated, particularly in the brain. However, how males and females with ASC are different from their respective typical controls are not always the same. In terms of brain morphometry, the SSE was a SSEMB pattern where an EMB pattern existed in females but not in males. Morphometric differences were much stronger between females with and without ASC, compared to that between the male groups. For brain oscillations, the SSE did not involve EMB pattern at all. The oscillation pattern differences were much stronger between males with and without ASC, compared to that between the female groups. Lastly, for brain regions showing SSE, they located differently in structural versus functional datasets. In sum, we observed an inconsistency of sex-ASC relationship at the brain level.

A possible explanation is that structural MRI measures very different properties from functional MRI. Furthermore we have examined only one (out of many) aspect that can be derived from structural MRI measurements (i.e., relative regional volume), and one (out of many) from functional MRI (i.e., signal complexity in terms of fractal geometry). Structural-functional correspondence is never straightforward. For example, one should not assume that a structure larger (or smaller) in size guarantees more (or less) regular intrinsic oscillations, or more (or less) activation in a particular cognitive task. Essentially they are about different properties of the brain. Unfortunately we are still not clear about the exact micro-neuroanatomical and physiological underpinnings of both the structural MRI volumetric measures (Kanai

& Rees, 2011) and the functional MRI oscillation complexity measures (Lai, et al., 2010; Maxim, et al., 2005). How exactly they may (or may not) associate with each other and to what extent their neurobiological underpinnings are mutually related await extensive fundamental studies. Based on these, it is understandable that the exact group difference patterns, effect sizes of group differences and regions showing SSE are all different when investigating the structural versus functional aspects of the brain.

Having said that, it is still worthwhile to speculate on how this seeming inconsistency arises. Brain volume is often considered an indicator of brain growth and remodelling (Giedd & Rapoport, 2010), therefore in adults it may be viewed as reflecting the outcome of growth and development, including experiential effects. It is dynamic from a longitudinal point of view, but usually does not show significant short-term change unless there is an acute pathological process ongoing (e.g. cerebral infarction). Therefore brain volume may be viewed as relatively stable in the short term, comparable to a *trait marker*. On the other hand, for resting-state spontaneous brain oscillations, though reflecting the default internal functional organisation (Greicius, 2008; Raichle et al., 2001) and correlating to structural connectivity (Greicius, et al., 2009; Honey, et al., 2009; Skudlarski, et al., 2008; van den Heuvel, et al., 2009), its complexity changes by cognitive load (Barnes, Bullmore, & Suckling, 2009). Therefore, resting brain oscillations may be more dynamic and reflects more of a here-and-now condition, comparable to a *state marker*. To interpret the seeming inconsistent SSE patterns between structural versus functional aspects by this viewpoint, the fact that female adults with ASC showed more evident structural differences from typical counterparts than that of male adults with ASC may indicate

a greater change in women with ASC in terms of brain growth and development. On the contrary, the fact that their current brain oscillation complexity was similar to typical women, whereas men with ASC showed a very different status from typical men, may indicate higher similarity of current brain functional status between females with and without ASC compared to that between the males groups.

In sum, sex-ASC relationship varies in the brain depending on whether the structural or functional aspect is under examination. The reason for this is still unknown but a speculation is that the former may act as a trait marker indicating results of development, whereas the latter a state marker indicating here-and-now functional status. A full picture delineating sex-ASC relationship in the brain requires multi-level (i.e., structural, functional, perfusion-wise, electrophysiological) and multi-aspect (i.e., different aspects within a level, e.g. both volumetric and shape measures for structure) approaches. The seeming inconsistency may just reflect the fact that the individual imaging method (and the aspect investigated) is only capable of illuminating part of the whole story.

7.2.3 How observations may inform potential mechanisms and future research directions

As pointed out in Chapter 1, the ultimate research concern on how sex relates to ASC is "*How does sex contribute to the aetiology and developmental mechanisms of ASC?*" The observational findings from this dissertation are not able to provide answers directly, but they facilitate hypothesis-generation for the mechanism aspects. Two speculations were proposed here, under the overarching theme of *sex-specific mechanisms or processes*.

7.2.3.1 Pretending to be normal?

The first speculation concerns sex-specific developmental changes of behaviour over time. As exemplified and discussed in detail in Chapter 2, male and female adults with ASC share similar childhood autistic symptom severity, but by adulthood females on average interact with people with less autistic characteristics compared to males (Lai, et al., 2011). In Chapter 3 we further demonstrated that female adults with ASC showed similarly poor social cognitive abilities as male adults with ASC. We suspect for women with ASC, the contrast between impaired social cognitive abilities and relatively intact interpersonal interaction skills is mainly due to their greater motivation and ability to *camouflage*, developed over the years. They may be able to mask their social communication difficulties by learning socially appropriate interaction behaviours and by constructing 'social scripts' to follow. However they strive to do this because the supporting social cognitive abilities are still relatively weak. It is also possible that although consciously 'pretending to be normal', they suffer from inabilities to react naturally, automatically and intuitively in social situations as typical people ordinarily do. We did not conduct formal qualitative research on this issue. However, in the interviews with care-givers when they were contrasting the child's childhood, teenage and adulthood, they often reported improved and *learned* social communication behaviours and skills over time, whilst constantly suffered from difficulties, failures and stress in nearly all social situations apart from being with immediate families. The same theme often revealed itself in the ADOS interviews when these women described current and past difficulties in their social life. It is worth noting that although we suspect this development of adaptive strategies is on average more evident in females, we do not preclude that males with

ASC are able to develop similar camouflage.

If this superficially quasi-normal social communication ability is afforded through their pretence to be normal, it is crucial to know how they accomplish this. *Do they rely on imitation, systemizing social and emotional themes, enhanced conscious mentalizing, repeated practice and rehearsal, or any other processes?* The second crucial question is about the motivation. *What drives them to tolerate the stress and uneasiness during social encounters? Is the motivation social in nature or associated with other rewards?* Clarification of the strategies they developed and employed and the underlying supportive motivations would be informative not only for understanding the mechanisms of change but also for designing intervention and supports. Third, how brain plasticity contributes is also central to understanding the changes in behaviour and cognition. *Are there neurobiological factors causally related to the development of adaptive strategies, or serve as the basis for staying motivated?* The last question regards the impacts and their best interests. *What are the potential side effects of camouflage? Are the co-occurring psychiatric symptoms related to this? Is it really the best strategy?* Though camouflage may work, the social encounters are always stressful and seldom pleasant for people with ASC. Therefore, it is important to understand the potential impacts to their mental well-being. We are not able to address these questions at this stage, but future studies using adequate research designs in proper settings will clarify how people with ASC modulate their behaviours in order to be socially adaptive, and what the resulting pros and cons are.

At least two research designs would address some of these queries. First, a retrospective, qualitative in-depth interview design will elicit much needed information on their own experiences growing up as people with ASC, especially if

being male or female makes a difference to the process. Qualitative research design and analysis strategies are capable of providing subjective recollections and reflections about the motivations, processes and strategies for change and adaptation, as well as subjective evaluations of the consequences. Second, a prospective, longitudinal follow-up design from early childhood to describe and delineate the sex-general and sex-specific developmental trajectories for social communication can provide objective observation and evaluation, as well as subjective reports. This can also help construct models to identify causal factors and modulators to the behavioural changes. How these factors and modulators influence social cognitive abilities, including both controlled and automatic processes, and whether the influences are sex-specific, are all key questions to address. How children with ASC interact with their environment, survive social demands and develop adaptive strategies will also be clarified in an ecologically valid manner. Neurobiological investigations will further the causal understandings to the brain. Lastly, from an intervention viewpoint, results from both research approaches may subserve the development of supports for people with ASC who still suffer from disabilities in social interactions and demands.

7.2.3.2 Masculinisation effect in brain anatomy?

The second speculation arises from the SSEMB pattern noted in brain structure. Since how ASC characterises the brain resembles masculinisation to a marked extent in females, by extrapolation it could be that biological processes relevant to masculinisation (i.e., sex differences) partially involve in the emergence of ASC in females. Sexual dimorphism in the brain can be influenced by both direct effects and interactive processes of hormone, sex chromosome genes and sex-specific

environment-mediated epigenetic effects (McCarthy & Arnold, 2011), as opposed to the traditional linear view of the hormone-mediated organisation and activation model. The emergence of ASC in females, again by extrapolation, may also involve the direct effects and interactions of all these factors. Our available data preliminarily suggested the potential role of prenatal androgen effect. But equally possible are the influences from sex chromosome genes, especially those on the X chromosome, as reviewed in Chapter 1 explaining the sex-differential liability of ASC. The emergence of ASC in females may therefore involve multiple additive or multiplicative processes, for example mutations on the X chromosome contributing to an increased vulnerability to prenatal androgen effect, further affected by multiple epigenetic modulations related to experiences and/or hormones (prenatal or postnatal). Sex-specific serum biomarker fingerprints for female adults with ASC is recently reported (Schwarz, et al., 2010), which includes mainly molecules related to growth factors and hormones (including androgens) but also some cytokine, inflammatory and other factors. This similarly indicates that although hormonal effect may play a crucial role, other biological factors may also have impacts. Though preliminary speculations, a wide range of hypotheses could be generated and tested in the future. One of the best designs will be a longitudinal study centred on these factors and processes, especially on potential sex-differential hormonal effects.

As mentioned earlier, we noted evident SSE in the brain. Potential sex-specific mechanisms leading up to a common presentation of core behavioural and cognitive features of ASC (i.e., *equifinality*) calls attention to start conducting mechanism- or process-oriented investigations delineating differential processes of the emergence of ASC in males and females. Whether the above-mentioned speculations for females

with ASC also apply to males with ASC awaits investigation. Clarifying potential sex-specific mechanisms leading up to or modifying the behavioural presentation of ASC should be one of the most important concerns for the field.

7.2.4 Practical implications for research and clinical services

Perhaps the most important practical implication for future research designs is that *autism research should stratify according to sex*. This has been claimed in the field of genetic research for ASC (Loat, et al., 2008) and should also be seriously considered for all levels of studies. By stratifying by sex it is plausible that the heterogeneity of ASC could be substantially clarified and better understood.

Clinically, the key message is that *sex differences in the presentation of ASC should be acknowledged*, to achieve a better recognition of women on the spectrum. Better recognition and diagnosis will hopefully facilitate sex-sensitive supports, acknowledging specific needs. Clinicians should be aware of the possible superficially able social communication abilities and camouflage. Information from multiple resources, including interview (and observation), self-reports, detailed developmental history and particularly neuropsychological assessments of social cognitive abilities should be routinely performed to assist diagnostic assessments.

Another issue relates to the identification and management of co-occurring psychiatric conditions (Tantam, 2000). In the present sample, male and female adults with ASC on average reported similar levels of anxiety, depression and obsessive-compulsive (OC) symptoms. However fewer males reported receiving medications for these conditions than females. Although self-reports may not properly reflect the clinical diagnoses, which require other detailed assessments, this observed

sex difference in receiving medical treatments suggests several possibilities that warrant clinical attention. It may be that for males and females with ASC who have similar levels of co-occurring anxiety, depression and OC symptoms, females are better recognised and treated. In other words, co-occurring symptoms in males may be under-recognised or even overlooked. It may also be that though reporting similar levels of symptoms, males and females show different severity or presentation of associated signs (e.g. physical agitation) that guide clinicians' different judgments on prescription. Research-wise this observation elicits interests in not only sex difference in co-occurring symptoms, but also in whether the corresponding services are delivered differentially. Clinically this reminds practitioners to carefully assess potential sex-specific presentations of co-occurring psychiatric conditions in male and female adults with ASC, and not to be biased by their own gender stereotype and/or stereotype to ASC on providing treatments and interventions.

7.3 Future Research Directions

As pointed out in Chapter 1, the major limitations of the studies arise from the sample selection strategy. The findings cannot be directly generalised to other subpopulations of ASC, e.g. people in difference age ranges (i.e., toddler, school-age children, adolescents or elderly people), early-diagnosed, having lower IQ or with medical or neurodevelopmental comorbidities. Furthermore, the observations in adulthood are inevitably a mixture of primary characteristics and secondary (and/or compensatory) changes. The next steps to further delineate how sex relates to ASC should aim at overcoming these limitations.

7.3.1 Future plans

First, a similar research framework can be applied to cross-sectional studies on other subpopulations in ASC. Of particular interest will be the younger populations, especially toddlers. This will bring us closer to the primary characteristics of ASC, with fewer confounds from experiential effects. Findings from different age ranges would help clarify how age and development modulate sex-ASC relationship and provide insights into potential developmental changes. Similarly, working with lower-IQ subpopulation will potentially delineate how general cognitive abilities influence sex-ASC relationship. All these strategies will contribute to completing the jigsaw of how sex relates to ASC, and how sex contributes to the emergence of ASC.

Second, one of the best strategies to address the mechanistic level questions is a longitudinal follow-up design. The analytical framework of this dissertation can be easily applied to ongoing baby-siblings follow-up projects, as well as any beginning cohort studies. Particular focus should be sex differences and similarities of the growth trajectories (biological, behavioural and cognitive) of male and female infants and toddlers who have ever shown early signs of autism. This will provide insights into early sex-general and sex-specific developmental trajectories related to ASC. Equally important is to characterise the developmental changes from middle childhood to adolescence for males and females with ASC, since this period is often socially challenging and demanding, and multiple sex-specific biological processes start working (e.g. the onset of puberty). From anecdotal reports of our participants, this period is one of the most difficult times for them but equally when most behavioural changes have occurred. Some start to experience the motivation to change their social communication style, to develop friendships and to acquire camouflage

strategies. It is particularly important to illuminate how these changes occur and strategies develop, what the short-term and long-term impacts are, and which associated neurobiological changes are involved. On causal investigation, it is also crucial to understand the roles of social environment and interpersonal interactions, life experiences, stress, motivation and puberty-related biological processes, to name a few. Longitudinal design is able to elucidate the developmental processes and shed light on the intervention and support strategies for ASC, from both sex-general and sex-specific perspectives.

Third, as proposed earlier, a retrospective, qualitative in-depth interview design is able to obtain much needed information on the subjective experiences growing up as an individual with ASC. It is important to understand the sex-general and sex-specific subjective experiences throughout development. This will complement the prospective, longitudinal follow-up design by providing qualitative characterisation of subjective experiences and self-appraisal, which is meanwhile informative to potential research focuses for the prospective studies.

Lastly, although the present participants are mainly young adults, it is possible to examine how age modulates sex-ASC relationship *in adulthood*, which is not a focus of this dissertation. Whether males or females, with or without ASC, have differential growth trajectories in adulthood is of interest for understanding life-span developments in ASC. For instance, differential age effects on brain structural changes have been demonstrated in men with and without ASC (Raznahan, Toro, et al., 2010). Whether this applies to females is unknown. One reason age effect was not examined in the present dissertation is because of the potentially insufficient sample size to provide adequate power to detect age-sex-ASC three-way interactions. We

have, however, initiated a consortium based on the AIMS protocol (i.e., the Female AIMS project) in order to double the sample size of the current cohort. Hopefully the effect of age can be then adequately illuminated, further clarifying another important aspect of the heterogeneity in ASC.

7.3.2 Projects in progress

A few projects based on this dissertation are starting to investigate the brain features from different perspectives and by different neuroimaging analytical methodologies. As mentioned in Chapters 4 and 5, VBM provides a non-biased morphometric investigation yet the multivariate patterns of the brain cannot be delineated. Surface-based morphometry is able to complement by characterising the geometric aspects. Graph theoretical approach treats the whole brain as a complex network (Bullmore & Bassett, 2011; Bullmore & Sporns, 2009), and can characterise functional correlation structure in resting-state fMRI or regional size covariance in structural MRI. This novel approach investigates the brain from a systems level rather than on local regions. Still another multivariate methodology, the machine-learning classifier approach (Pereira, Mitchell, & Botvinick, 2009), leads to a plausible avenue incorporating brain measures in predicting diagnostic status (Bosl, Tierney, Tager-Flusberg, & Nelson, 2011; Ecker, Marquand, et al., 2010; Ecker, Rocha-Rego, et al., 2010). This could be applied to both the structural and functional datasets to examine if it accurately discriminates typical males from females, as well as between diagnostic groups in either sex or across sex. Advances in these examinations may help validate the classifier approach and explore its potential usage in delineating the heterogeneity of ASC, especially sex-related, at the brain level. Finally, we have also

collected diffusion imaging for all participants, which is capable of characterising the white matter microstructural properties. Given that we found significant SSEMB pattern particularly on the relative volume of the white matter, it is crucial to further examine if the same pattern appears in microstructural properties. This will provide further insights to the neurobiological nature of the noted SSEMB pattern.

Second, the SSEMB pattern in brain morphometry could be partially related to (prenatal) hormonal mechanisms. Examining the relationship between current hormonal status and current brain properties may provide additional looks to the issue. Given recent reports on group differences in current serum hormonal concentrations (Ruta, et al., 2011) and sex-specific biomarkers involving hormones (Schwarz, et al., 2010), to clarify how current hormonal status correlates with brain structure and function may shed light on the underlying interactive processes contributing to the emergence of ASC. We have collected salivary sample for all participants so this analysis will be attainable.

Lastly, one missing piece of the whole picture is the genetic and epigenetic aspects. For the current sample we have also collected DNA. This provides with the unique opportunity to investigate how epi/genetic markers correlate with brain characteristics, and to explore if the correlation is expressed differentially by sex. Sex chromosome characteristics, including genetic markers in DNA sequence (e.g. single nucleotide polymorphism or copy number variations) and indicators of epigenetic influences (e.g. DNA methylation), are of particular interests given the hypotheses outlined in Chapter 1.

7.4 Concluding Remarks

ASC is a heterogeneous condition. A thorough understanding to ASC is not attainable unless the heterogeneity is carefully delineated. One of the pivots is sex. There has been heightened awareness in the field of neuroscience on the differences and similarities between sexes regarding how they affect typical molecular processes, neurophysiology and neuroanatomy (Cahill, 2006; McCarthy & Arnold, 2011). This dissertation furthers the scope to an atypical condition, ASC, by showing the crucial role of sex at the behavioural, cognitive, brain structural and functional levels. Studying sex differences facilitates understanding to the causal mechanisms of psychopathology (Rutter, et al., 2003). Studying sex differences and similarities *within* ASC not only helps delineate the heterogeneity, facilitates awareness to the sex-general and sex-specific characteristics, but also paves a path to illuminate the emergence of ASC.

Sex matters in autism, but how it matters is complicated. We hope the studies have provided the initial steps to fill the knowledge gap and will remind people of the importance of both sex similarities and differences within ASC.

References

Abell, F., Krams, M., Ashburner, J., Passingham, R., Friston, K., Frackowiak, R., et al. (1999). The neuroanatomy of autism: a voxel-based whole brain analysis of structural scans. *Neuroreport, 10*(8), 1647-1651.

Abrahams, B. S., & Geschwind, D. H. (2008). Advances in autism genetics: on the threshold of a new neurobiology. *Nat Rev Genet, 9*(5), 341-355.

Adolphs, R. (2009). The social brain: neural basis of social knowledge. *Annu Rev Psychol, 60*, 693-716.

Aleman, A., & Swart, M. (2008). Sex differences in neural activation to facial expressions denoting contempt and disgust. *PLoS ONE, 3*(11), e3622.

Allison, C., Baron-Cohen, S., Wheelwright, S., Charman, T., Richler, J., Pasco, G., et al. (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. *J Autism Dev Disord, 38*(8), 1414-1425.

Amaral, D. G., Schumann, C. M., & Nordahl, C. W. (2008). Neuroanatomy of autism. *Trends Neurosci, 31*(3), 137-145.

American Psychiatric Association. (1980). *Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (DSM-III)*. Washington, DC: American Psychiatric Publishing, Inc.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders, 4th edition text revision (DSM-IV-TR)*. Washington, DC: American Psychiatric Publishing, Inc.

American Psychiatric Association. (2011). DSM-5 Development: Proposed Revision-Autism Spectrum Disorder. Retrieved June 3, 2011, from http://www.dsm5.org/ProposedRevisions/Pages/proposedrevision.aspx?rid=94#

Amodio, D. M., & Frith, C. D. (2006). Meeting of minds: the medial frontal cortex and social cognition. *Nat Rev Neurosci, 7*(4), 268-277.

Ankney, C. D. (1992). The brain size/IQ debate. *Nature, 360*(6402), 292.

Armstrong, E., Schleicher, A., Omran, H., Curtis, M., & Zilles, K. (1995). The ontogeny of human gyrification. *Cereb Cortex, 5*(1), 56-63.

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *Neuroimage, 38*(1), 95-113.

Ashburner, J., & Friston, K. J. (2000). Voxel-based morphometry--the methods. *Neuroimage, 11*(6 Pt 1), 805-821.

Ashwin, C., Baron-Cohen, S., Wheelwright, S., O'Riordan, M., & Bullmore, E. T. (2007). Differential activation of the amygdala and the 'social brain' during fearful face-processing in Asperger Syndrome. *Neuropsychologia, 45*(1), 2-14.

Ashwin, C., Chapman, E., Colle, L., & Baron-Cohen, S. (2006). Impaired recognition of negative basic emotions in autism: a test of the amygdala theory. *Soc Neurosci, 1*(3-4), 349-363.

Ashwin, C., Wheelwright, S., & Baron-Cohen, S. (2006). Attention bias to faces in Asperger Syndrome: a pictorial emotion Stroop study. *Psychol Med, 36*(6), 835-843.

Asperger, H. (1944). Die "autistichen psychopathen" im kindersalter [Autistic psychopathy in childhood]. *Archive fur Psychiatrie und Nervenkrankheiten, 117*, 76-136.

Assadi, S. M., Yucel, M., & Pantelis, C. (2009). Dopamine modulates neural networks involved in effort-based decision-making. *Neurosci Biobehav Rev, 33*(3), 383-393.

Attwood, T. (2006). The pattern of abilities and development of girls with Asperger's syndrome. *Asperger's and girls.* Arlington, TX: Future Horisons, Inc.

Attwood, T. (2007). *The complete guide to Asperger's syndrome.* London, UK: Jessica Kingsley Publishers.

Attwood, T., & Grandin, T. (2006). *Asperger's and girls*. Arlington, TX: Future Horizons, Inc.

Auyeung, B., Baron-Cohen, S., Ashwin, E., Knickmeyer, R., Taylor, K., & Hackett, G. (2009). Fetal testosterone and autistic traits. *Br J Psychol, 100*(Pt 1), 1-22.

Auyeung, B., Baron-Cohen, S., Chapman, E., Knickmeyer, R., Taylor, K., & Hackett, G. (2006). Foetal testosterone and the child systemizing quotient. *European Journal of Endocrinology, 155*(suppl_1), S123.

Auyeung, B., Baron-Cohen, S., Wheelwright, S., & Allison, C. (2008). The Autism Spectrum Quotient: Children's Version (AQ-Child). *J Autism Dev Disord, 38*(7), 1230-1240.

Auyeung, B., Taylor, K., Hackett, G., & Baron-Cohen, S. (2010). Foetal testosterone and autistic traits in 18 to 24-month-old children. *Mol Autism, 1*(1), 11.

Auyeung, B., Wheelwright, S., Allison, C., Atkinson, M., Samarawickrema, N., & Baron-Cohen, S. (2009). The children's Empathy Quotient and Systemizing

Quotient: sex differences in typical development and in Autism Spectrum Conditions. *J Autism Dev Disord, 39*(11), 1509-1521.

Aylward, E. H., Minshew, N. J., Field, K., Sparks, B. F., & Singh, N. (2002). Effects of age on brain volume and head circumference in autism. *Neurology, 59*(2), 175-183.

Bagby, R. M., Parker, J. D., & Taylor, G. J. (1994). The twenty-item Toronto Alexithymia Scale--I. Item selection and cross-validation of the factor structure. *J Psychosom Res, 38*(1), 23-32.

Baird, G., Charman, T., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., et al. (2008). Regression, developmental trajectory and associated problems in disorders in the autism spectrum: the SNAP study. *J Autism Dev Disord, 38*(10), 1827-1836.

Baird, G., Simonoff, E., Pickles, A., Chandler, S., Loucas, T., Meldrum, D., et al. (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*(9531), 210-215.

Banach, R., Thompson, A., Szatmari, P., Goldberg, J., Tuff, L., Zwaigenbaum, L., et al. (2009). Brief Report: Relationship between non-verbal IQ and gender in autism. *J Autism Dev Disord, 39*(1), 188-193.

Bao, A. M., & Swaab, D. F. (2010). Sex differences in the brain, behavior, and neuropsychiatric disorders. *Neuroscientist, 16*(5), 550-565.

Barnes, A., Bullmore, E. T., & Suckling, J. (2009). Endogenous human brain dynamics recover slowly following cognitive effort. *PLoS ONE, 4*(8), e6626.

Baron-Cohen, S. (1995). *Mindblindness: An essay on autism and theory of mind*. Boston: MIT Press/Bradford Books.

Baron-Cohen, S. (2000). Is asperger syndrome/high-functioning autism necessarily a disability? *Dev Psychopathol, 12*(3), 489-500.

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

Baron-Cohen, S. (2003). *The essential difference.* New York, NY: Basic Books.

Baron-Cohen, S. (2008). *Autism and Asperger syndrome: The facts.* Oxford, UK: Oxford University Press.

Baron-Cohen, S. (2010). Empathizing, systemizing, and the extreme male brain theory of autism. *Prog Brain Res, 186*, 167-175.

Baron-Cohen, S., Ashwin, E., Ashwin, C., Tavassoli, T., & Chakrabarti, B. (2009). Talent in autism: hyper-systemizing, hyper-attention to detail and sensory hypersensitivity. *Philos Trans R Soc Lond B Biol Sci, 364*(1522), 1377-1383.

Baron-Cohen, S., & Belmonte, M. K. (2005). Autism: a window onto the development of the social and the analytic brain. *Annu Rev Neurosci, 28*, 109-126.

Baron-Cohen, S., Hoekstra, R. A., Knickmeyer, R., & Wheelwright, S. (2006). The Autism-Spectrum Quotient (AQ)--adolescent version. *J Autism Dev Disord, 36*(3), 343-350.

Baron-Cohen, S., Knickmeyer, R. C., & Belmonte, M. K. (2005). Sex differences in the brain: implications for explaining autism. *Science, 310*(5749), 819-823.

Baron-Cohen, S., Leslie, A. M., & Frith, U. (1985). Does the autistic child have a "theory of mind"? *Cognition, 21*(1), 37-46.

Baron-Cohen, S., Lombardo, M. V., Auyeung, B., Ashwin, E., Chakrabarti, B., & Knickmeyer, R. (2011). Why are autism spectrum conditions more prevalent in males? *PLoS Biol, 9*(6), e1001081.

Baron-Cohen, S., Richler, J., Bisarya, D., Gurunathan, N., & Wheelwright, S. (2003). The systemizing quotient: an investigation of adults with Asperger syndrome or high-functioning autism, and normal sex differences. *Philos Trans R Soc Lond B Biol Sci, 358*(1430), 361-374.

Baron-Cohen, S., Ring, H., Chitnis, X., Wheelwright, S., Gregory, L., Williams, S., et al. (2006). fMRI of parents of children with Asperger Syndrome: a pilot study. *Brain Cogn, 61*(1), 122-130.

Baron-Cohen, S., Ring, H., Moriarty, J., Schmitz, B., Costa, D., & Ell, P. (1994). Recognition of mental state terms. Clinical findings in children with autism and a functional neuroimaging study of normal adults. *Br J Psychiatry, 165*(5), 640-649.

Baron-Cohen, S., Ring, H. A., Bullmore, E. T., Wheelwright, S., Ashwin, C., & Williams, S. C. (2000). The amygdala theory of autism. *Neurosci Biobehav Rev, 24*(3), 355-364.

Baron-Cohen, S., Scott, F. J., Allison, C., Williams, J., Bolton, P., Matthews, F. E., et al. (2009). Prevalence of autism-spectrum conditions: UK school-based population study. *Br J Psychiatry, 194*(6), 500-509.

Baron-Cohen, S., & Wheelwright, S. (2004). The empathy quotient: an investigation of adults with Asperger syndrome or high functioning autism, and normal sex differences. *J Autism Dev Disord, 34*(2), 163-175.

Baron-Cohen, S., Wheelwright, S., Hill, J., Raste, Y., & Plumb, I. (2001). The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J Child Psychol Psychiatry, 42*(2), 241-251.

Baron-Cohen, S., Wheelwright, S., Robinson, J., & Woodbury-Smith, M. (2005). The Adult Asperger Assessment (AAA): a diagnostic method. *J Autism Dev Disord, 35*(6), 807-819.

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. *J Autism Dev Disord, 31*(1), 5-17.

Bastiaansen, J. A., Meffert, H., Hein, S., Huizinga, P., Ketelaars, C., Pijnenborg, M., et al. (2010). Diagnosing autism spectrum disorders in adults: the use of Autism Diagnostic Observation Schedule (ADOS) module 4. *J Autism Dev Disord*.

Bauman, M. L., & Kemper, T. L. (2005). Neuroanatomic observations of the brain in autism: a review and future directions. *Int J Dev Neurosci, 23*(2-3), 183-187.

Beaudet, A. L. (2007). Autism: highly heritable but not inherited. *Nat Med, 13*(5), 534-536.

Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An inventory for measuring clinical anxiety: Psychometric properties. *Journal of Consulting and clinical Psychology, 56*(6), 893-897.

Beck, A. T., Ward, C. H., Mendelson, M. L., Mock, J. E., & Erbaugh, J. K. (1961). An inventory for measuring depression. *Arch Gen Psychiatry, 4*(6), 561.

Beckers, F., Verheyden, B., & Aubert, A. E. (2006). Aging and nonlinear heart rate control in a healthy population. *Am J Physiol Heart Circ Physiol, 290*(6), H2560-2570.

Beckers, F., Verheyden, B., Couckuyt, K., & Aubert, A. E. (2006). Fractal dimension in health and heart failure. *Biomed Tech (Berl), 51*(4), 194-197.

Behrens, T. E., Hunt, L. T., & Rushworth, M. F. (2009). The computation of social behavior. *Science, 324*(5931), 1160-1164.

Behrens, T. E., Hunt, L. T., Woolrich, M. W., & Rushworth, M. F. (2008). Associative learning of social value. *Nature, 456*(7219), 245-249.

Behrens, T. E., Johansen-Berg, H., Woolrich, M. W., Smith, S. M., Wheeler-Kingshott, C. A., Boulby, P. A., et al. (2003). Non-invasive mapping of connections between human thalamus and cortex using diffusion imaging. *Nat Neurosci, 6*(7), 750-757.

Behrens, T. E., Woolrich, M. W., Walton, M. E., & Rushworth, M. F. (2007). Learning the value of information in an uncertain world. *Nat Neurosci, 10*(9), 1214-1221.

Belmonte, M. K., Allen, G., Beckel-Mitchener, A., Boulanger, L. M., Carper, R. A., &

Webb, S. J. (2004). Autism and abnormal development of brain connectivity. *J Neurosci, 24*(42), 9228-9231.

Ben-Sasson, A., Hen, L., Fluss, R., Cermak, S. A., Engel-Yeger, B., & Gal, E. (2009). A meta-analysis of sensory modulation symptoms in individuals with autism spectrum disorders. *J Autism Dev Disord, 39*(1), 1-11.

Ben Shalom, D. (2000). Autism: Emotions without feelings. *Autism, 4*(2), 205-207.

Berah, E. F. (1983). Sex differences in psychiatric morbidity: an analysis of Victorian data. *Aust N Z J Psychiatry, 17*(3), 266-273.

Biswal, B. B., Mennes, M., Zuo, X. N., Gohel, S., Kelly, C., Smith, S. M., et al. (2010). Toward discovery science of human brain function. *Proc Natl Acad Sci U S A, 107*(10), 4734-4739.

Blakemore, S. J. (2008). The social brain in adolescence. *Nat Rev Neurosci, 9*(4), 267-277.

Bloss, C. S., & Courchesne, E. (2007). MRI neuroanatomy in young girls with autism: a preliminary study. *J Am Acad Child Adolesc Psychiatry, 46*(4), 515-523.

Boddaert, N., Chabane, N., Gervais, H., Good, C. D., Bourgeois, M., Plumet, M. H., et al. (2004). Superior temporal sulcus anatomical abnormalities in childhood autism: a voxel-based morphometry MRI study. *Neuroimage, 23*(1), 364-369.

Bolte, S., Duketis, E., Poustka, F., & Holtmann, M. (2011). Sex differences in cognitive domains and their clinical correlates in higher-functioning autism spectrum disorders. *Autism, 15*(4), 497-511.

Bolton, P. F., Macdonald, H., Pickles, A., Rios, P., Goode, S., Crowson, M., et al. (1994). A case-control family history study of autism. *J Child Psychol Psychiatry, 35*(5), 877-900.

Bolton, P. F., Pickles, A., Murphy, M., & Rutter, M. (1998). Autism, affective and other psychiatric disorders: patterns of familial aggregation. *Psychol Med, 28*(2), 385-395.

Bonilha, L., Cendes, F., Rorden, C., Eckert, M., Dalgalarrondo, P., Li, L. M., et al. (2008). Gray and white matter imbalance--typical structural abnormality underlying classic autism? *Brain Dev, 30*(6), 396-401.

Bosl, W., Tierney, A., Tager-Flusberg, H., & Nelson, C. (2011). EEG complexity as a biomarker for autism spectrum disorder risk. *BMC Med, 9*, 18.

Botvinick, M., Nystrom, L. E., Fissell, K., Carter, C. S., & Cohen, J. D. (1999). Conflict monitoring versus selection-for-action in anterior cingulate cortex. *Nature, 402*(6758), 179-181.

Boucher, J. (2009). *The autistic spectrum: Characteristics, causes and practical issues*. London, UK: SAGE Publications Ltd.

Boutin, P., Maziade, M., Merette, C., Mondor, M., Bedard, C., & Thivierge, J. (1997). Family history of cognitive disabilities in first-degree relatives of autistic and mentally retarded children. *J Autism Dev Disord, 27*(2), 165-176.

Brambilla, P., Hardan, A., di Nemi, S. U., Perez, J., Soares, J. C., & Barale, F. (2003). Brain anatomy and development in autism: review of structural MRI studies. *Brain Res Bull, 61*(6), 557-569.

Breedlove, S. M. (2010). Minireview: Organizational hypothesis: instances of the fingerpost. *Endocrinology, 151*(9), 4116-4122.

Brieber, S., Neufang, S., Bruning, N., Kamp-Becker, I., Remschmidt, H., Herpertz-Dahlmann, B., et al. (2007). Structural brain abnormalities in adolescents with autism spectrum disorder and patients with attention deficit/hyperactivity disorder. *J Child Psychol Psychiatry, 48*(12), 1251-1258.

Brock, J., Brown, C. C., Boucher, J., & Rippon, G. (2002). The temporal binding deficit hypothesis of autism. *Dev Psychopathol, 14*(2), 209-224.

Brugha, T. S., McManus, S., Bankart, J., Scott, F., Purdon, S., Smith, J., et al. (2011). Epidemiology of autism spectrum disorders in adults in the community in England. *Arch Gen Psychiatry, 68*(5), 459-465.

Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. L. (2008). The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci, 1124*, 1-38.

Buckner, R. L., & Carroll, D. C. (2007). Self-projection and the brain. *Trends Cogn Sci, 11*(2), 49-57.

Buckner, R. L., Sepulcre, J., Talukdar, T., Krienen, F. M., Liu, H., Hedden, T., et al. (2009). Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer's disease. *J Neurosci, 29*(6), 1860-1873.

Bullmore, E., Barnes, A., Bassett, D. S., Fornito, A., Kitzbichler, M., Meunier, D., et al. (2009). Generic aspects of complexity in brain imaging data and other biological systems. *Neuroimage, 47*(3), 1125-1134.

Bullmore, E., & Bassett, D. S. (2011). Brain graphs: graphical models of the human brain connectome. *Annu Rev Clin Psychol, 7*, 113-140.

Bullmore, E., Brammer, M. J., Bourlon, P., Alarcon, G., Polkey, C. E., Elwes, R., et al. (1994). Fractal analysis of electroencephalographic signals intracerebrally recorded during 35 epileptic seizures: evaluation of a new method for synoptic visualisation of ictal events. *Electroencephalogr Clin Neurophysiol, 91*(5), 337-345.

Bullmore, E., Fadili, J., Breakspear, M., Salvador, R., Suckling, J., & Brammer, M.

(2003). Wavelets and statistical analysis of functional magnetic resonance images of the human brain. *Stat Methods Med Res, 12*(5), 375-399.

Bullmore, E., Fadili, J., Maxim, V., Sendur, L., Whitcher, B., Suckling, J., et al. (2004). Wavelets and functional magnetic resonance imaging of the human brain. *Neuroimage, 23 Suppl 1*, S234-249.

Bullmore, E., & Sporns, O. (2009). Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci, 10*(3), 186-198.

Bullmore, E., Suckling, J., Overmeyer, S., Rabe-Hesketh, S., Taylor, E., & Brammer, M. J. (1999). Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Trans Med Imaging, 18*(1), 32-42.

Bush, G., Vogt, B. A., Holmes, J., Dale, A. M., Greve, D., Jenike, M. A., et al. (2002). Dorsal anterior cingulate cortex: a role in reward-based decision making. *Proc Natl Acad Sci U S A, 99*(1), 523-528.

Cahill, L. (2006). Why sex matters for neuroscience. *Nat Rev Neurosci, 7*(6), 477-484.

Campbell, R., Elgar, K., Kuntsi, J., Akers, R., Terstegge, J., Coleman, M., et al. (2002). The classification of 'fear' from faces is associated with face recognition skill in women. *Neuropsychologia, 40*(6), 575-584.

Carter, A. S., Black, D. O., Tewani, S., Connolly, C. E., Kadlec, M. B., & Tager-Flusberg, H. (2007). Sex differences in toddlers with autism spectrum disorders. *J Autism Dev Disord, 37*(1), 86-97.

Casanova, M. F., van Kooten, I. A., Switala, A. E., van Engeland, H., Heinsen, H., Steinbusch, H. W., et al. (2006). Minicolumnar abnormalities in autism. *Acta Neuropathol (Berl), 112*(3), 287-303.

Castelli, F., Frith, C., Happe, F., & Frith, U. (2002). Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. *Brain, 125*(Pt 8), 1839-1849.

Chapman, E., Baron-Cohen, S., Auyeung, B., Knickmeyer, R., Taylor, K., & Hackett, G. (2006). Fetal testosterone and empathy: evidence from the empathy quotient (EQ) and the "reading the mind in the eyes" test. *Soc Neurosci, 1*(2), 135-148.

Charman, T., Jones, C. R., Pickles, A., Simonoff, E., Baird, G., & Happe, F. (2011). Defining the cognitive phenotype of autism. *Brain Res, 1380*, 10-21.

Chen, R., Jiao, Y., & Herskovits, E. H. (2011). Structural MRI in autism spectrum disorder. *Pediatr Res, 69*(5 Pt 2), 63R-68R.

Chen, X., Sachdev, P. S., Wen, W., & Anstey, K. J. (2007). Sex differences in regional

gray matter in healthy individuals aged 44-48 years: a voxel-based morphometric study. *Neuroimage, 36*(3), 691-699.

Cheng, Y., Chou, K. H., Decety, J., Chen, I. Y., Hung, D., Tzeng, O. J., et al. (2009). Sex differences in the neuroanatomy of human mirror-neuron system: a voxel-based morphometric investigation. *Neuroscience, 158*(2), 713-720.

Cheng, Y., Chou, K. H., Fan, Y. T., & Lin, C. P. (2011). ANS: Aberrant Neurodevelopment of the Social Cognition Network in Adolescents with Autism Spectrum Disorders. *PLoS ONE, 6*(4), e18905.

Chumbley, J. R., & Friston, K. J. (2009). False discovery rate revisited: FDR and topological inference using Gaussian random fields. *Neuroimage, 44*(1), 62-70.

Cicchetti, D. (2006). Development and psychopathology. In D. Cicchetti & D. J. Cohen (Eds.), *Developmental Psychopathology. Volume 1: Theory and method.* (2nd ed.). Hoboken, NJ: John Wiley & Sons, Inc.

Clark, A. S., MacLusky, N. J., & Goldman-Rakic, P. S. (1988). Androgen binding and metabolism in the cerebral cortex of the developing rhesus monkey. *Endocrinology, 123*(2), 932-940.

Clark, D. L., Boutros, N. N., & Mendez, M. F. (2010). *The brain and behavior: An introduction to behavioral neuroanatomy* (3rd ed.). Cambridge, UK: Cambridge University Press.

Clements-Stephens, A. M., Rimrodt, S. L., & Cutting, L. E. (2009). Developmental sex differences in basic visuospatial processing: differences in strategy use? *Neurosci Lett, 449*(3), 155-160.

Cloutier, J., Heatherton, T. F., Whalen, P. J., & Kelley, W. M. (2008). Are attractive people rewarding? Sex differences in the neural substrates of facial attractiveness. *J Cogn Neurosci, 20*(6), 941-951.

Connell, R. (2009). *Gender.* (2nd ed.). Cambridge, UK: Polity Press.

Constantino, J. N. (2011). The quantitative nature of autistic social impairment. *Pediatr Res, 69*(5 Pt 2), 55R-62R.

Constantino, J. N., Przybeck, T., Friesen, D., & Todd, R. D. (2000). Reciprocal social behavior in children with and without pervasive developmental disorders. *J Dev Behav Pediatr, 21*(1), 2-11.

Constantino, J. N., & Todd, R. D. (2003). Autistic traits in the general population: a twin study. *Arch Gen Psychiatry, 60*(5), 524-530.

Corden, B., Chilvers, R., & Skuse, D. (2008). Avoidance of emotionally arousing stimuli predicts social-perceptual impairment in Asperger's syndrome. *Neuropsychologia, 46*(1), 137-147.

Cosgrove, K. P., Mazure, C. M., & Staley, J. K. (2007). Evolving knowledge of sex differences in brain structure, function, and chemistry. *Biol Psychiatry, 62*(8), 847-855.

Courchesne, E., Campbell, K., & Solso, S. (2011). Brain growth across the life span in autism: age-specific changes in anatomical pathology. *Brain Res, 1380*, 138-145.

Courchesne, E., Carper, R., & Akshoomoff, N. (2003). Evidence of brain overgrowth in the first year of life in autism. *Jama, 290*(3), 337-344.

Courchesne, E., & Pierce, K. (2005). Brain overgrowth in autism during a critical time in development: implications for frontal pyramidal neuron and interneuron development and connectivity. *Int J Dev Neurosci, 23*(2-3), 153-170.

Courchesne, E., Pierce, K., Schumann, C. M., Redcay, E., Buckwalter, J. A., Kennedy, D. P., et al. (2007). Mapping early brain development in autism. *Neuron, 56*(2), 399-413.

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nat Rev Neurosci, 3*(8), 655-666.

Craig, M. C., Zaman, S. H., Daly, E. M., Cutter, W. J., Robertson, D. M., Hallahan, B., et al. (2007). Women with autistic-spectrum disorder: magnetic resonance imaging study of brain anatomy. *Br J Psychiatry, 191*, 224-228.

Crick, N. R., & Zahn-Waxler, C. (2003). The development of psychopathology in females and males: current progress and future challenges. *Dev Psychopathol, 15*(3), 719-742.

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., & Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nat Neurosci, 7*(2), 189-195.

Cunningham, W. A., Johnson, M. K., Raye, C. L., Chris Gatenby, J., Gore, J. C., & Banaji, M. R. (2004). Separable neural components in the processing of black and white faces. *Psychol Sci, 15*(12), 806-813.

Damoiseaux, J. S., Rombouts, S. A., Barkhof, F., Scheltens, P., Stam, C. J., Smith, S. M., et al. (2006). Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A, 103*(37), 13848-13853.

Davis, G., & Plaisted, K. (2007). Autism: not interested or not 'tuned-in'? *Curr Biol, 17*(19), R851-853.

Dawood, M. Y. (1977). Hormones in amniotic fluid. *Am J Obstet Gynecol, 128*(5), 576-583.

Dawson, G., Toth, K., Abbott, R., Osterling, J., Munson, J., Estes, A., et al. (2004). Early social attention impairments in autism: social orienting, joint attention,

and attention to distress. *Dev Psychol, 40*(2), 271-283.

De Bruin, E. I., De Nijs, P. F., Verheij, F., Verhagen, D. H., & Ferdinand, R. F. (2009). Autistic features in girls from a psychiatric sample are strongly associated with a low 2D:4D ratio. *Autism, 13*(5), 511-521.

De Luca, M., Beckmann, C. F., De Stefano, N., Matthews, P. M., & Smith, S. M. (2006). fMRI resting state networks define distinct modes of long-distance interactions in the human brain. *Neuroimage, 29*(4), 1359-1367.

DeMyer, M. K., Alpern, G. D., Barton, S., DeMyer, W. E., Churchill, D. W., Hingtgen, J. N., et al. (1972). Imitation in autistic, early schizophrenic, and non-psychotic subnormal children. *J Autism Child Schizophr, 2*(3), 264-287.

Deoni, S. C., Williams, S. C., Jezzard, P., Suckling, J., Murphy, D. G., & Jones, D. K. (2008). Standardized structural magnetic resonance imaging in multicentre studies using quantitative T1 and T2 imaging at 1.5 T. *Neuroimage, 40*(2), 662-671.

Department of Health, U. K. (2010). *Fulfilling and rewarding lives: The strategy for adults with autism in England.*

Di Martino, A., Shehzad, Z., Kelly, C., Roy, A. K., Gee, D. G., Uddin, L. Q., et al. (2009). Relationship between cingulo-insular functional connectivity and autistic traits in neurotypical adults. *Am J Psychiatry, 166*(8), 891-899.

Draganski, B., Gaser, C., Busch, V., Schuierer, G., Bogdahn, U., & May, A. (2004). Neuroplasticity: changes in grey matter induced by training. *Nature, 427*(6972), 311-312.

Driemeyer, J., Boyke, J., Gaser, C., Buchel, C., & May, A. (2008). Changes in gray matter induced by learning--revisited. *PLoS ONE, 3*(7), e2669.

Duman, R. S. (2009). Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. *Dialogues Clin Neurosci, 11*(3), 239-255.

Dumontheil, I., Hassan, B., Gilbert, S. J., & Blakemore, S. J. (2010). Development of the selection and manipulation of self-generated thoughts in adolescence. *J Neurosci, 30*(22), 7664-7671.

Ecker, C., Marquand, A., Mourao-Miranda, J., Johnston, P., Daly, E. M., Brammer, M. J., et al. (2010). Describing the brain in autism in five dimensions--magnetic resonance imaging-assisted diagnosis of autism spectrum disorder using a multiparameter classification approach. *J Neurosci, 30*(32), 10612-10623.

Ecker, C., Rocha-Rego, V., Johnston, P., Mourao-Miranda, J., Marquand, A., Daly, E. M., et al. (2010). Investigating the predictive value of whole-brain structural MR scans in autism: a pattern classification approach. *Neuroimage, 49*(1),

44-56.

Ecker, C., Suckling, J., Deoni, S., Lombardo, M. V., Bullmore, E., Baron-Cohen, S., et al. (in press). Brain anatomy and its relationship to behavior in adults with autism: A multi-centre MRI study. *Arch Gen Psychiatry*.

Eme, R. F. (1992). Selective females affliction in the developmental disorders of childhood: A literature review. *Journal of Clinical Child & Adolescent Psychology, 21*(4), 354-364.

Ernsperger, L., & Wendel, D. (2007). *Girls under the umbrella of autism spectrum disorders.* Shawnee Mission, KS: Autism Asperger Publishing Company.

Fair, D. A., Cohen, A. L., Dosenbach, N. U., Church, J. A., Miezin, F. M., Barch, D. M., et al. (2008). The maturing architecture of the brain's default network. *Proc Natl Acad Sci U S A, 105*(10), 4028-4032.

Fair, D. A., Cohen, A. L., Power, J. D., Dosenbach, N. U., Church, J. A., Miezin, F. M., et al. (2009). Functional brain networks develop from a "local to distributed" organization. *PLoS Comput Biol, 5*(5), e1000381.

Fair, D. A., Dosenbach, N. U., Church, J. A., Cohen, A. L., Brahmbhatt, S., Miezin, F. M., et al. (2007). Development of distinct control networks through segregation and integration. *Proc Natl Acad Sci U S A, 104*(33), 13507-13512.

Fein, D., Pennington, B., Markowitz, P., Braverman, M., & Waterhouse, L. (1986). Toward a neuropsychological model of infantile autism: are the social deficits primary? *J Am Acad Child Psychiatry, 25*(2), 198-212.

Finegan, J. A., Bartleman, B., & Wong, P. Y. (1989). A window for the study of prenatal sex hormone influences on postnatal development. *J Genet Psychol, 150*(1), 101-112.

Fisher, R. A. (1921). On the probable error of a coefficient of correlation deduced from a small sample. *Metron, 1*, 3-32.

Fleming, S. M., Weil, R. S., Nagy, Z., Dolan, R. J., & Rees, G. (2010). Relating introspective accuracy to individual differences in brain structure. *Science, 329*(5998), 1541-1543.

Foa, E. B., Huppert, J. D., Leiberg, S., Langner, R., Kichic, R., Hajcak, G., et al. (2002). The Obsessive-Compulsive Inventory: development and validation of a short version. *Psychol Assess, 14*(4), 485-496.

Fombonne, E. (2003). Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev Disord, 33*(4), 365-382.

Fombonne, E. (2005). Epidemiological studies of pervasive developmental disorder. In F. Volkmar, R. Paul, A. Klin & D. Cohen (Eds.), *Handbook of Autism and Pervasive Developmental Disorders* (pp. 42-69). Hoboken, NJ: Wiley.

Fornito, A., & Bullmore, E. T. (2010). What can spontaneous fluctuations of the blood oxygenation-level-dependent signal tell us about psychiatric disorders? *Curr Opin Psychiatry, 23*(3), 239-249.

Fournier, K. A., Hass, C. J., Naik, S. K., Lodha, N., & Cauraugh, J. H. (2010). Motor coordination in autism spectrum disorders: a synthesis and meta-analysis. *J Autism Dev Disord, 40*(10), 1227-1240.

Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci, 8*(9), 700-711.

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., & Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A, 102*(27), 9673-9678.

Fransson, P., Skiold, B., Horsch, S., Nordell, A., Blennow, M., Lagercrantz, H., et al. (2007). Resting-state networks in the infant brain. *Proc Natl Acad Sci U S A, 104*(39), 15531-15536.

Freitag, C. M., Konrad, C., Haberlen, M., Kleser, C., von Gontard, A., Reith, W., et al. (2008). Perception of biological motion in autism spectrum disorders. *Neuropsychologia, 46*(5), 1480-1494.

Frith, C. D. (2007). The social brain? *Philos Trans R Soc Lond B Biol Sci, 362*(1480), 671-678.

Frith, U. (1989). *Autism: Explaining the enigma*. Oxford, UK: Blackwell.

Frith, U. (2001). Mind blindness and the brain in autism. *Neuron, 32*(6), 969-979.

Frith, U. (Ed.). (1991). *Autism and Asperger syndrome.* Cambridge, UK: Cambridge University Press.

Gao, W., Zhu, H., Giovanello, K. S., Smith, J. K., Shen, D., Gilmore, J. H., et al. (2009). Evidence on the emergence of the brain's default network from 2-week-old to 2-year-old healthy pediatric subjects. *Proc Natl Acad Sci U S A, 106*(16), 6790-6795.

Garn, S. M., Burdi, A. R., Babler, W. J., & Stinson, S. (1975). Early prenatal attainment of adult metacarpal-phalangeal rankings and proportions. *Am J Phys Anthropol, 43*(3), 327-332.

Gathercole, S. E., Willis, C. S., Baddeley, A. D., & Emslie, H. (1994). The Children's Test of Nonword Repetition: a test of phonological working memory. *Memory, 2*(2), 103-127.

Gendry Meresse, I., Zilbovicius, M., Boddaert, N., Robel, L., Philippe, A., Sfaello, I., et al. (2005). Autism severity and temporal lobe functional abnormalities. *Ann*

Neurol, 58(3), 466-469.

Geschwind, D. H., & Levitt, P. (2007). Autism spectrum disorders: developmental disconnection syndromes. *Curr Opin Neurobiol, 17*(1), 103-111.

Geschwind, N., & Levitsky, W. (1968). Human brain: left-right asymmetries in temporal speech region. *Science, 161*(837), 186-187.

Geurts, H. M., Corbett, B., & Solomon, M. (2009). The paradox of cognitive flexibility in autism. *Trends Cogn Sci, 13*(2), 74-82.

Geurts, H. M., & Vissers, M. E. (2011). Elderly with autism: executive functions and memory. *J Autism Dev Disord*.

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., et al. (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nat Neurosci, 2*(10), 861-863.

Giedd, J. N., & Rapoport, J. L. (2010). Structural MRI of pediatric brain development: what have we learned and where are we going? *Neuron, 67*(5), 728-734.

Gillberg, C. (2002). *A Guide to Asperger Syndrome.* Cambridge, UK: Cambridge University Press.

Gillberg, C., & Coleman, M. (2000). *The biology of the autistic syndromes.* (3rd ed.). Cambridge, UK: Cambridge University Press.

Gilmore, J. H., Lin, W., Prastawa, M. W., Looney, C. B., Vetsa, Y. S., Knickmeyer, R. C., et al. (2007). Regional gray matter growth, sexual dimorphism, and cerebral asymmetry in the neonatal brain. *J Neurosci, 27*(6), 1255-1260.

Glass, G. V., Peckham, P. D., & Sanders, J. R. (1972). Consequences of failure to meet assumptions underlying the fixed effects analyses of variance and covariance. *Review of Educational Research, 42*(3), 237-288.

Goin-Kochel, R. P., Abbacchi, A., & Constantino, J. N. (2007). Lack of evidence for increased genetic loading for autism among families of affected females: a replication from family history data in two large samples. *Autism, 11*(3), 279-286.

Golan, O., & Baron-Cohen, S. (2006). Systemizing empathy: teaching adults with Asperger syndrome or high-functioning autism to recognize complex emotions using interactive multimedia. *Dev Psychopathol, 18*(2), 591-617.

Golarai, G., Grill-Spector, K., & Reiss, A. L. (2006). Autism and the development of face processing. *Clin Neurosci Res, 6*(3), 145-160.

Goldberger, A. L., Amaral, L. A., Hausdorff, J. M., Ivanov, P., Peng, C. K., & Stanley, H. E. (2002). Fractal dynamics in physiology: alterations with disease and aging. *Proc Natl Acad Sci U S A, 99 Suppl 1*, 2466-2472.

Goldenfeld, N., Baron-Cohen, S., & Wheelwright, S. (2005). Empathizing and

systemizing in males, females and autism. *Clinical Neuropsychiatry, 2*(6), 338-345.

Goldstein, J. M., Seidman, L. J., Horton, N. J., Makris, N., Kennedy, D. N., Caviness, V. S., Jr., et al. (2001). Normal sexual dimorphism of the adult human brain assessed by in vivo magnetic resonance imaging. *Cereb Cortex, 11*(6), 490-497.

Good, C. D., Johnsrude, I., Ashburner, J., Henson, R. N., Friston, K. J., & Frackowiak, R. S. (2001). Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. *Neuroimage, 14*(3), 685-700.

Gotham, K., Pickles, A., & Lord, C. (2009). Standardizing ADOS scores for a measure of severity in autism spectrum disorders. *J Autism Dev Disord, 39*(5), 693-705.

Gotham, K., Risi, S., Pickles, A., & Lord, C. (2007). The Autism Diagnostic Observation Schedule: revised algorithms for improved diagnostic validity. *J Autism Dev Disord, 37*(4), 613-627.

Grafton, S. T., Mazziotta, J. C., Woods, R. P., & Phelps, M. E. (1992). Human functional anatomy of visually guided finger movements. *Brain, 115 (Pt 2)*, 565-587.

Grandin, T., & Scariano, M. M. (1996). *Emergence: Labeled autistic*. New York, NY: Warner Books, Inc.

Green, D., Charman, T., Pickles, A., Chandler, S., Loucas, T., Simonoff, E., et al. (2009). Impairment in movement skills of children with autistic spectrum disorders. *Dev Med Child Neurol, 51*(4), 311-316.

Green, D. M., & Swets, J. A. (1966). *Signal detection theory and psychophysics*. New York, NY: Wiley.

Greicius, M. D. (2008). Resting-state functional connectivity in neuropsychiatric disorders. *Curr Opin Neurol, 21*(4), 424-430.

Greicius, M. D., Supekar, K., Menon, V., & Dougherty, R. F. (2009). Resting-state functional connectivity reflects structural connectivity in the default mode network. *Cereb Cortex, 19*(1), 72-78.

Grelotti, D. J., Gauthier, I., & Schultz, R. T. (2002). Social interest and the development of cortical face specialization: what autism teaches us about face processing. *Dev Psychobiol, 40*(3), 213-225.

Gribbin, J. (2005). *Deep simplicity: Chaos, complexity and the emergence of life*. London, UK: Penguin.

Hadjikhani, N., Joseph, R. M., Manoach, D. S., Naik, P., Snyder, J., Dominick, K., et

al. (2009). Body expressions of emotion do not trigger fear contagion in autism spectrum disorder. *Soc Cogn Affect Neurosci, 4*(1), 70-78.

Hadjikhani, N., Joseph, R. M., Snyder, J., & Tager-Flusberg, H. (2006). Anatomical differences in the mirror neuron system and social cognition network in autism. *Cereb Cortex, 16*(9), 1276-1282.

Hagemann, G., Ugur, T., Schleussner, E., Mentzel, H. J., Fitzek, C., Witte, O. W., et al. (2011). Changes in brain size during the menstrual cycle. *PLoS ONE, 6*(2), e14655.

Hagmann, P., Cammoun, L., Gigandet, X., Meuli, R., Honey, C. J., Wedeen, V. J., et al. (2008). Mapping the structural core of human cerebral cortex. *PLoS Biol, 6*(7), e159.

Hallahan, B., Daly, E. M., McAlonan, G., Loth, E., Toal, F., O'Brien, F., et al. (2009). Brain morphometry volume in autistic spectrum disorder: a magnetic resonance imaging study of adults. *Psychol Med, 39*(2), 337-346.

Halpern, D. F. (2000). *Sex differences in cognitive abilities.* (3rd ed.). London, UK: Lawrence Earlbaum.

Hamilton, C. (2008). *Cognition and sex differences.* Houndmills, UK: Palgrave MacMillan.

Happe, F. (1999). Autism: cognitive deficit or cognitive style? *Trends Cogn Sci, 3*(6), 216-222.

Happe, F. (2011). Criteria, categories, and continua: autism and related disorders in DSM-5. *J Am Acad Child Adolesc Psychiatry, 50*(6), 540-542.

Happe, F., & Frith, U. (2006). The weak coherence account: detail-focused cognitive style in autism spectrum disorders. *J Autism Dev Disord, 36*(1), 5-25.

Hardan, A. Y., Girgis, R. R., Adams, J., Gilbert, A. R., Keshavan, M. S., & Minshew, N. J. (2006). Abnormal brain size effect on the thalamus in autism. *Psychiatry Res, 147*(2-3), 145-151.

Hardan, A. Y., Minshew, N. J., Melhem, N. M., Srihari, S., Jo, B., Bansal, R., et al. (2008). An MRI and proton spectroscopy study of the thalamus in children with autism. *Psychiatry Res, 163*(2), 97-105.

Harms, M. B., Martin, A., & Wallace, G. L. (2010). Facial emotion recognition in autism spectrum disorders: a review of behavioral and neuroimaging studies. *Neuropsychol Rev, 20*(3), 290-322.

Hartley, S. L., & Sikora, D. M. (2009). Sex differences in autism spectrum disorder: an examination of developmental functioning, autistic symptoms, and coexisting behavior problems in toddlers. *J Autism Dev Disord, 39*(12), 1715-1722.

Hassabis, D., & Maguire, E. A. (2007). Deconstructing episodic memory with construction. *Trends Cogn Sci, 11*(7), 299-306.

Haznedar, M. M., Buchsbaum, M. S., Hazlett, E. A., LiCalzi, E. M., Cartwright, C., & Hollander, E. (2006). Volumetric analysis and three-dimensional glucose metabolic mapping of the striatum and thalamus in patients with autism spectrum disorders. *Am J Psychiatry, 163*(7), 1252-1263.

Haznedar, M. M., Buchsbaum, M. S., Metzger, M., Solimando, A., Spiegel-Cohen, J., & Hollander, E. (1997). Anterior cingulate gyrus volume and glucose metabolism in autistic disorder. *Am J Psychiatry, 154*(8), 1047-1050.

Henley, S. M. D., Ridgway, G. R., Scahill, R. I., Kloppel, S., Tabrizi, S. J., Fox, N. C., et al. (2010). Pitfalls in the use of voxel-based morphometry as a biomarker: examples from Huntington disease. *American Journal of Neuroradiology, 31*(4), 711.

Herbert, M. R., Ziegler, D. A., Makris, N., Filipek, P. A., Kemper, T. L., Normandin, J. J., et al. (2004). Localization of white matter volume increase in autism and developmental language disorder. *Ann Neurol, 55*(4), 530-540.

Herman, P., Kocsis, L., & Eke, A. (2009). Fractal characterization of complexity in dynamic signals: application to cerebral hemodynamics. *Methods Mol Biol, 489*, 23-40.

Hermelin, B., & O'Connor, N. (1985). Logico-affective states and non-verbal language. In E. Schopler & G. Mesibov (Eds.), *Communication problems in autism.* (pp. 293-309). New York, NY: Plenum Press.

Hernandez, N., Metzger, A., Magne, R., Bonnet-Brilhault, F., Roux, S., Barthelemy, C., et al. (2009). Exploration of core features of a human face by healthy and autistic adults analyzed by visual scanning. *Neuropsychologia, 47*(4), 1004-1012.

Hill, E. L. (2004a). Evaluating the theory of executive dysfunction in autism. *Developmental Review, 24*(2), 189-233.

Hill, E. L. (2004b). Executive dysfunction in autism. *Trends Cogn Sci, 8*(1), 26-32.

Hines, M. (2005). *Brain gender.* New York, NY: Oxford University Press.

Hines, M. (2010). Sex-related variation in human behavior and the brain. *Trends Cogn Sci, 14*(10), 448-456.

Hobson, R. P. (1986). The autistic child's appraisal of expressions of emotion. *J Child Psychol Psychiatry, 27*(3), 321-342.

Hobson, R. P. (1993). *Autism and the development of mind*. Hillsdale, NJ: Erlbaum.

Hobson, R. P. (2002). *The cradle of thought: Exploring the origins of thinking*. London: Macmillan.

Hobson, R. P., & Lee, A. (1999). Imitation and identification in autism. *J Child Psychol Psychiatry, 40*(4), 649-659.

Hobson, R. P., & Meyer, J. (2006). Imitation, identification, and the shaping of mind: Insights from autism. In S. J. Rogers & J. H. G. Williams (Eds.), *Imitation and the social mind: Autism and typical development* (pp. 198-224). New York: The Guilford Press.

Hofvander, B., Delorme, R., Chaste, P., Nyden, A., Wentz, E., Stahlberg, O., et al. (2009). Psychiatric and psychosocial problems in adults with normal-intelligence autism spectrum disorders. *BMC Psychiatry, 9*, 35.

Hollander, E., Anagnostou, E., Chaplin, W., Esposito, K., Haznedar, M. M., Licalzi, E., et al. (2005). Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. *Biol Psychiatry, 58*(3), 226-232.

Holtmann, M., Bolte, S., & Poustka, F. (2007). Autism spectrum disorders: sex differences in autistic behaviour domains and coexisting psychopathology. *Dev Med Child Neurol, 49*(5), 361-366.

Honey, C. J., Sporns, O., Cammoun, L., Gigandet, X., Thiran, J. P., Meuli, R., et al. (2009). Predicting human resting-state functional connectivity from structural connectivity. *Proc Natl Acad Sci U S A, 106*(6), 2035-2040.

Howard, M. A., Cowell, P. E., Boucher, J., Broks, P., Mayes, A., Farrant, A., et al. (2000). Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. *Neuroreport, 11*(13), 2931-2935.

Humphreys, K., Minshew, N., Leonard, G. L., & Behrmann, M. (2007). A fine-grained analysis of facial expression processing in high-functioning adults with autism. *Neuropsychologia, 45*(4), 685-695.

Huttenlocher, P. R. (1990). Morphometric study of human cerebral cortex development. *Neuropsychologia, 28*(6), 517-527.

Hyde, J. S. (2005). The gender similarities hypothesis. *Am Psychol, 60*(6), 581-592.

Hyde, J. S. (2007). New directions in the study of gender similarities and differences. *Current Directions in Psychological Science, 16*(5), 259-263.

Hyde, J. S., Fennema, E., & Lamon, S. J. (1990). Gender differences in mathematics performance: a meta-analysis. *Psychol Bull, 107*(2), 139-155.

Hyde, K. L., Samson, F., Evans, A. C., & Mottron, L. (2010). Neuroanatomical differences in brain areas implicated in perceptual and other core features of autism revealed by cortical thickness analysis and voxel-based morphometry. *Hum Brain Mapp, 31*(4), 556-566.

Iacoboni, M., & Dapretto, M. (2006). The mirror neuron system and the consequences of its dysfunction. *Nat Rev Neurosci, 7*(12), 942-951.

Iarocci, G., & McDonald, J. (2006). Sensory integration and the perceptual experience of persons with autism. *J Autism Dev Disord, 36*(1), 77-90.

Im, K., Lee, J. M., Lee, J., Shin, Y. W., Kim, I. Y., Kwon, J. S., et al. (2006). Gender difference analysis of cortical thickness in healthy young adults with surface-based methods. *Neuroimage, 31*(1), 31-38.

Insel, T. R. (2010). Faulty circuits. *Sci Am, 302*(4), 44-51.

Jacquemont, M. L., Sanlaville, D., Redon, R., Raoul, O., Cormier-Daire, V., Lyonnet, S., et al. (2006). Array-based comparative genomic hybridisation identifies high frequency of cryptic chromosomal rearrangements in patients with syndromic autism spectrum disorders. *J Med Genet, 43*(11), 843-849.

Jansiewicz, E. M., Goldberg, M. C., Newschaffer, C. J., Denckla, M. B., Landa, R., & Mostofsky, S. H. (2006). Motor signs distinguish children with high functioning autism and Asperger's syndrome from controls. *J Autism Dev Disord, 36*(5), 613-621.

Jarrold, C., Gilchrist, I. D., & Bender, A. (2005). Embedded figures detection in autism and typical development: preliminary evidence of a double dissociation in relationships with visual search. *Dev Sci, 8*(4), 344-351.

Jeste, S. S. (2011). The neurology of autism spectrum disorders. *Curr Opin Neurol, 24*(2), 132-139.

Jolliffe, T., & Baron-Cohen, S. (1997). Are people with autism and Asperger syndrome faster than normal on the Embedded Figures Test? *J Child Psychol Psychiatry, 38*(5), 527-534.

Jones, C. R., Pickles, A., Falcaro, M., Marsden, A. J., Happe, F., Scott, S. K., et al. (2011). A multimodal approach to emotion recognition ability in autism spectrum disorders. *J Child Psychol Psychiatry, 52*(3), 275-285.

Jones, W., & Klin, A. (2009). Heterogeneity and homogeneity across the autism spectrum: the role of development. *J Am Acad Child Adolesc Psychiatry, 48*(5), 471-473.

Jordan-Young, R. M. (2010). *Brain storm: The flaws in the science of sex differences*. Cambridge, MA: Harvard University Press.

Just, M. A., Cherkassky, V. L., Keller, T. A., & Minshew, N. J. (2004). Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain, 127*(Pt 8), 1811-1821.

Kaland, N., Mortensen, E. L., & Smith, L. (2007). Disembedding performance in children and adolescents with Asperger syndrome or high-functioning autism. *Autism, 11*(1), 81-92.

Kana, R. K., Keller, T. A., Cherkassky, V. L., Minshew, N. J., & Adam Just, M. (2008). Atypical frontal-posterior synchronization of Theory of Mind regions in autism during mental state attribution. *Soc Neurosci*, 1-18.

Kanai, R., & Rees, G. (2011). The structural basis of inter-individual differences in human behaviour and cognition. *Nat Rev Neurosci, 12*(4), 231-242.

Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child, 2*, 217-250.

Ke, X., Hong, S., Tang, T., Zou, B., Li, H., Hang, Y., et al. (2008). Voxel-based morphometry study on brain structure in children with high-functioning autism. *Neuroreport, 19*(9), 921-925.

Ke, X., Tang, T., Hong, S., Hang, Y., Zou, B., Li, H., et al. (2009). White matter impairments in autism, evidence from voxel-based morphometry and diffusion tensor imaging. *Brain Res, 1265*, 171-177.

Kennedy, D. P., & Courchesne, E. (2008). The intrinsic functional organization of the brain is altered in autism. *Neuroimage, 39*(4), 1877-1885.

Kimura, D. (1999). *Sex and cognition.* Cambridge, MA: The MIT Press.

Kitzbichler, M. G., Smith, M. L., Christensen, S. R., & Bullmore, E. (2009). Broadband criticality of human brain network synchronization. *PLoS Comput Biol, 5*(3), e1000314.

Klin, A., Jones, W., Schultz, R., & Volkmar, F. (2003). The enactive mind, or from actions to cognition: lessons from autism. *Philos Trans R Soc Lond B Biol Sci, 358*(1430), 345-360.

Knickmeyer, R. C., Baron-Cohen, S., Raggatt, P., & Taylor, K. (2005). Foetal testosterone, social relationships, and restricted interests in children. *J Child Psychol Psychiatry, 46*(2), 198-210.

Knickmeyer, R. C., Baron-Cohen, S., Raggatt, P., Taylor, K., & Hackett, G. (2006). Fetal testosterone and empathy. *Horm Behav, 49*(3), 282-292.

Knickmeyer, R. C., Woolson, S., Hamer, R. M., Konneker, T., & Gilmore, J. H. (2011). 2D:4D ratios in the first 2years of life: Stability and relation to testosterone exposure and sensitivity. *Horm Behav*.

Kolb, B., & Stewart, J. (1991). Sex-related differences in dendritic branching of cells in the prefrontal cortex of rats. *J Neuroendocrinol, 3*(1), 95-99.

Kopp, S., & Gillberg, C. (1992). Girls with social deficits and learning problems: Autism, atypical Asperger syndrome or a variant of these conditions. *European Child & Adolescent Psychiatry, 1*(2), 89-99.

Kopp, S., & Gillberg, C. (2011). The Autism Spectrum Screening Questionnaire (ASSQ)-Revised Extended Version (ASSQ-REV): An instrument for better

capturing the autism phenotype in girls? A preliminary study involving 191 clinical cases and community controls. *Res Dev Disabil*.

Kosaka, H., Omori, M., Munesue, T., Ishitobi, M., Matsumura, Y., Takahashi, T., et al. (2010). Smaller insula and inferior frontal volumes in young adults with pervasive developmental disorders. *Neuroimage, 50*(4), 1357-1363.

Koscik, T., O'Leary, D., Moser, D. J., Andreasen, N. C., & Nopoulos, P. (2009). Sex differences in parietal lobe morphology: relationship to mental rotation performance. *Brain Cogn, 69*(3), 451-459.

Koyama, T., Kamio, Y., Inada, N., & Kurita, H. (2009). Sex differences in WISC-III profiles of children with high-functioning pervasive developmental disorders. *J Autism Dev Disord, 39*(1), 135-141.

Krach, S., Blumel, I., Marjoram, D., Lataster, T., Krabbendam, L., Weber, J., et al. (2009). Are women better mindreaders? Sex differences in neural correlates of mentalizing detected with functional MRI. *BMC Neurosci, 10*, 9.

Krebs, J. F., Biswas, A., Pascalis, O., Kamp-Becker, I., Remschmidt, H., & Schwarzer, G. (2011). Face processing in children with autism spectrum disorder: independent or interactive processing of facial identity and facial expression? *J Autism Dev Disord, 41*(6), 796-804.

Kurth, F., Narr, K. L., Woods, R. P., O'Neill, J., Alger, J. R., Caplan, R., et al. (2011). Diminished gray matter within the hypothalamus in autism disorder: a potential link to hormonal effects? *Biol Psychiatry, 70*(3), 278-282.

Kwon, H., Ow, A. W., Pedatella, K. E., Lotspeich, L. J., & Reiss, A. L. (2004). Voxel-based morphometry elucidates structural neuroanatomy of high-functioning autism and Asperger syndrome. *Dev Med Child Neurol, 46*(11), 760-764.

Lai, M. C., Lombardo, M. V., Chakrabarti, B., Sadek, S. A., Pasco, G., Wheelwright, S. J., et al. (2010). A shift to randomness of brain oscillations in people with autism. *Biol Psychiatry, 68*(12), 1092-1099.

Lai, M. C., Lombardo, M. V., Pasco, G., Ruigrok, A. N., Wheelwright, S. J., Sadek, S. A., et al. (2011). A behavioral comparison of male and female adults with high functioning autism spectrum conditions. *PLoS ONE, 6*(6), e20835.

Langen, M., Schnack, H. G., Nederveen, H., Bos, D., Lahuis, B. E., de Jonge, M. V., et al. (2009). Changes in the developmental trajectories of striatum in autism. *Biol Psychiatry, 66*(4), 327-333.

Law Smith, M. J., Montagne, B., Perrett, D. I., Gill, M., & Gallagher, L. (2010). Detecting subtle facial emotion recognition deficits in high-functioning Autism using dynamic stimuli of varying intensities. *Neuropsychologia, 48*(9),

2777-2781.

Le Couteur, A., Rutter, M., Lord, C., Rios, P., Robertson, S., Holdgrafer, M., et al. (1989). Autism diagnostic interview: a standardized investigator-based instrument. *J Autism Dev Disord, 19*(3), 363-387.

Lemon, J. M., Gargaro, B., Enticott, P. G., & Rinehart, N. J. (2011). Executive functioning in autism spectrum disorders: a gender comparison of response inhibition. *J Autism Dev Disord, 41*(3), 352-356.

Lenroot, R. K., Gogtay, N., Greenstein, D. K., Wells, E. M., Wallace, G. L., Clasen, L. S., et al. (2007). Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *Neuroimage, 36*(4), 1065-1073.

Leopold, D. A. (2009). Neuroscience: Pre-emptive blood flow. *Nature, 457*(7228), 387-388.

Lin, L. Y., Lin, J. L., Du, C. C., Lai, L. P., Tseng, Y. Z., & Huang, S. K. (2001). Reversal of deteriorated fractal behavior of heart rate variability by beta-blocker therapy in patients with advanced congestive heart failure. *J Cardiovasc Electrophysiol, 12*(1), 26-32.

Linkenkaer-Hansen, K., Nikouline, V. V., Palva, J. M., & Ilmoniemi, R. J. (2001). Long-range temporal correlations and scaling behavior in human brain oscillations. *J Neurosci, 21*(4), 1370-1377.

Llinas, R., Ribary, U., Contreras, D., & Pedroarena, C. (1998). The neuronal basis for consciousness. *Philos Trans R Soc Lond B Biol Sci, 353*(1377), 1841-1849.

Loat, C. S., Asbury, K., Galsworthy, M. J., Plomin, R., & Craig, I. W. (2004). X inactivation as a source of behavioural differences in monozygotic female twins. *Twin Res, 7*(1), 54-61.

Loat, C. S., Haworth, C. M., Plomin, R., & Craig, I. W. (2008). A model incorporating potential skewed X-inactivation in MZ girls suggests that X-linked QTLs exist for several social behaviours including autism spectrum disorder. *Ann Hum Genet, 72*(Pt 6), 742-751.

Lombardo, M. V., Barnes, J. L., Wheelwright, S. J., & Baron-Cohen, S. (2007). Self-referential cognition and empathy in autism. *PLoS ONE, 2*(9), e883.

Lombardo, M. V., & Baron-Cohen, S. (2011). The role of the self in mindblindness in autism. *Conscious Cogn, 20*(1), 130-140.

Lombardo, M. V., Baron-Cohen, S., Belmonte, M. K., & Chakrabarti, B. (2009). Neural endophenotypes for social behaviour in autism spectrum conditions. In J. Decety & J. Cacioppo (Eds.), *Handbook of Social Neuroscience*. Oxford, UK: Oxford University Press.

Lombardo, M. V., Chakrabarti, B., Bullmore, E. T., Sadek, S. A., Pasco, G.,

Wheelwright, S. J., et al. (2010). Atypical neural self-representation in autism. *Brain, 133*(Pt 2), 611-624.

Lombardo, M. V., Chakrabarti, B., Bullmore, E. T., Wheelwright, S. J., Sadek, S. A., Suckling, J., et al. (2010). Shared neural circuits for mentalizing about the self and others. *J Cogn Neurosci, 22*(7), 1623-1635.

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Jr., Leventhal, B. L., DiLavore, P. C., et al. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord, 30*(3), 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. *J Autism Dev Disord, 24*(5), 659-685.

Lord, C., & Schopler, E. (1985). Differences in sex ratios in autism as a function of measured intelligence. *J Autism Dev Disord, 15*(2), 185-193.

Lord, C., Schopler, E., & Revicki, D. (1982). Sex differences in autism. *J Autism Dev Disord, 12*(4), 317-330.

Losh, M., Adolphs, R., Poe, M. D., Couture, S., Penn, D., Baranek, G. T., et al. (2009). Neuropsychological profile of autism and the broad autism phenotype. *Arch Gen Psychiatry, 66*(5), 518-526.

Lotspeich, L. J., Kwon, H., Schumann, C. M., Fryer, S. L., Goodlin-Jones, B. L., Buonocore, M. H., et al. (2004). Investigation of neuroanatomical differences between autism and Asperger syndrome. *Arch Gen Psychiatry, 61*(3), 291-298.

Lotter, V. (1966). Epidemiology of autistic conditions in young children. *Social Psychiatry and Psychiatric Epidemiology, 1*(3), 124-135.

Loucas, T., Riches, N. G., Charman, T., Pickles, A., Simonoff, E., Chandler, S., et al. (2010). Speech perception and phonological short-term memory capacity in language impairment: preliminary evidence from adolescents with specific language impairment (SLI) and autism spectrum disorders (ASD). *Int J Lang Commun Disord, 45*(3), 275-286.

Loveland, K. A. (2001). Toward an ecological theory of autism. In J. A. Burack, T. Charman, N. Yirmiya & P. R. Zelazo (Eds.), *The development of autism: Perspectives from theory and research.* (pp. 17-38). Mahwah, NJ: Lawrence Erlbaum Associates.

Luders, E., Gaser, C., Narr, K. L., & Toga, A. W. (2009). Why sex matters: brain size independent differences in gray matter distributions between men and women. *J Neurosci, 29*(45), 14265-14270.

Luders, E., Narr, K. L., Thompson, P. M., Rex, D. E., Woods, R. P., Deluca, H., et al. (2006). Gender effects on cortical thickness and the influence of scaling. *Hum Brain Mapp, 27*(4), 314-324.

Luders, E., Narr, K. L., Thompson, P. M., Woods, R. P., Rex, D. E., Jancke, L., et al. (2005). Mapping cortical gray matter in the young adult brain: effects of gender. *Neuroimage, 26*(2), 493-501.

Luders, E., Thompson, P. M., Narr, K. L., Toga, A. W., Jancke, L., & Gaser, C. (2006). A curvature-based approach to estimate local gyrification on the cortical surface. *Neuroimage, 29*(4), 1224-1230.

Luders, E., & Toga, A. W. (2010). Sex differences in brain anatomy. *Prog Brain Res, 186*, 3-12.

Lugnegard, T., Hallerback, M. U., & Gillberg, C. (2011). Psychiatric comorbidity in young adults with a clinical diagnosis of Asperger syndrome. *Res Dev Disabil, 32*(5), 1910-1917.

Lundqvist, D., Flykt, A., & Ohman, A. (1998). *The Karolinska Directed Emotional Faces - KDEF.* Stockholm, Sweden: Psychology Section, Department of Clinical Neuroscience, Karolinska Institute.

Lutchmaya, S., Baron-Cohen, S., & Raggatt, P. (2001). Foetal testosterone and vocabulary size in 18-and 24-month-old infants. *Infant Behavior and Development, 24*(4), 418-424.

Lutchmaya, S., Baron-Cohen, S., & Raggatt, P. (2002). Foetal testosterone and eye contact in 12-month-old human infants. *Infant Behavior and Development, 25*(3), 327-335.

Lutchmaya, S., Baron-Cohen, S., Raggatt, P., Knickmeyer, R., & Manning, J. T. (2004). 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Hum Dev, 77*(1-2), 23-28.

Macintosh, K. E., & Dissanayake, C. (2004). Annotation: The similarities and differences between autistic disorder and Asperger's disorder: a review of the empirical evidence. *J Child Psychol Psychiatry, 45*(3), 421-434.

Maguire, E. A., Gadian, D. G., Johnsrude, I. S., Good, C. D., Ashburner, J., Frackowiak, R. S., et al. (2000). Navigation-related structural change in the hippocampi of taxi drivers. *Proc Natl Acad Sci U S A, 97*(8), 4398-4403.

Mandelbrot, B. B. (1977). *The fractal geometry of nature.* New York, NY: WH Freeman.

Manning, J. T., Baron-Cohen, S., Wheelwright, S., & Sanders, G. (2001). The 2nd to 4th digit ratio and autism. *Dev Med Child Neurol, 43*(3), 160-164.

Manning, J. T., Bundred, P. E., & Flanagan, B. F. (2002). The ratio of 2nd to 4th digit

length: a proxy for transactivation activity of the androgen receptor gene? *Med Hypotheses, 59*(3), 334-336.

Manning, J. T., Bundred, P. E., Newton, D. J., & Flanagan, B. F. (2003). The second to fourth digit ratio and variation in the androgen receptor gene. *Evolution and Human Behavior, 24*(6), 399-405.

Manning, J. T., Fink, B., Neave, N., & Caswell, N. (2005). Photocopies yield lower digit ratios (2D:4D) than direct finger measurements. *Arch Sex Behav, 34*(3), 329-333.

Manning, J. T., Scutt, D., Wilson, J., & Lewis-Jones, D. I. (1998). The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Hum Reprod, 13*(11), 3000-3004.

Mari, M., Castiello, U., Marks, D., Marraffa, C., & Prior, M. (2003). The reach-to-grasp movement in children with autism spectrum disorder. *Philos Trans R Soc Lond B Biol Sci, 358*(1430), 393-403.

Markram, H., Rinaldi, T., & Markram, K. (2007). The intense world syndrome--an alternative hypothesis for autism. *Front Neurosci, 1*(1), 77-96.

Maxim, V., Sendur, L., Fadili, J., Suckling, J., Gould, R., Howard, R., et al. (2005). Fractional Gaussian noise, functional MRI and Alzheimer's disease. *Neuroimage, 25*(1), 141-158.

McAlonan, G. M., Cheung, C., Cheung, V., Wong, N., Suckling, J., & Chua, S. E. (2009). Differential effects on white-matter systems in high-functioning autism and Asperger's syndrome. *Psychol Med, 39*(11), 1885-1893.

McAlonan, G. M., Cheung, V., Cheung, C., Suckling, J., Lam, G. Y., Tai, K. S., et al. (2005). Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. *Brain, 128*(Pt 2), 268-276.

McAlonan, G. M., Daly, E., Kumari, V., Critchley, H. D., van Amelsvoort, T., Suckling, J., et al. (2002). Brain anatomy and sensorimotor gating in Asperger's syndrome. *Brain, 125*(Pt 7), 1594-1606.

McAlonan, G. M., Suckling, J., Wong, N., Cheung, V., Lienenkaemper, N., Cheung, C., et al. (2008). Distinct patterns of grey matter abnormality in high-functioning autism and Asperger's syndrome. *J Child Psychol Psychiatry, 49*(12), 1287-1295.

McCarthy, M. M., & Arnold, A. P. (2011). Reframing sexual differentiation of the brain. *Nat Neurosci, 14*(6), 677-683.

McLennan, J. D., Lord, C., & Schopler, E. (1993). Sex differences in higher functioning people with autism. *J Autism Dev Disord, 23*(2), 217-227.

Mesibov, G. B., Schopler, E., Schaffer, B., & Michal, N. (1989). Use of the childhood autism rating scale with autistic adolescents and adults. *J Am Acad Child Adolesc Psychiatry, 28*(4), 538-541.

Miller, J. K. (2003). *Women from another planet? Our lives in the universe of autism.* Bloomington, IN: 1st Books Library.

Minshew, N. J., Webb, S. J., Williams, D. L., & Dawson, G. (2006). Neuropsychology and neurophysiology of autism spectrum disorders. In S. O. Moldin & J. L. Rubenstein (Eds.), *Understanding autism: From basic neuroscience to treatment* (pp. 379-415). Boca Raton, FL: Taylor & Francis Group.

Mizuno, A., Villalobos, M. E., Davies, M. M., Dahl, B. C., & Muller, R. A. (2006). Partially enhanced thalamocortical functional connectivity in autism. *Brain Res, 1104*(1), 160-174.

Monk, C. S., Peltier, S. J., Wiggins, J. L., Weng, S. J., Carrasco, M., Risi, S., et al. (2009). Abnormalities of intrinsic functional connectivity in autism spectrum disorders. *Neuroimage, 47*(2), 764-772.

Monteggia, L. M., & Kavalali, E. T. (2009). Rett syndrome and the impact of MeCP2 associated transcriptional mechanisms on neurotransmission. *Biol Psychiatry, 65*(3), 204-210.

Moran, J. M., Young, L. L., Saxe, R., Lee, S. M., O'Young, D., Mavros, P. L., et al. (2011). Impaired theory of mind for moral judgment in high-functioning autism. *Proc Natl Acad Sci U S A, 108*(7), 2688-2692.

Morcom, A. M., & Fletcher, P. C. (2007). Does the brain have a baseline? Why we should be resisting a rest. *Neuroimage, 37*(4), 1073-1082.

Morton, J. (2004). *Understanding developmental disorders: A causal modelling approach.* Oxford, UK: Blackwell Publishing.

Mosconi, M. W., Cody-Hazlett, H., Poe, M. D., Gerig, G., Gimpel-Smith, R., & Piven, J. (2009). Longitudinal study of amygdala volume and joint attention in 2- to 4-year-old children with autism. *Arch Gen Psychiatry, 66*(5), 509-516.

Mottron, L., & Burack, J. A. (2001). Enhanced perceptual functioning in the development of autism. In J. A. Burack, T. Charman, N. Yirmiya & P. R. Zelazo (Eds.), *The development of autism: Perspectives from theory and research.* (pp. 131-148). Mahwah, NJ: Lawrence Erlbaum Associates.

Mottron, L., Dawson, M., Soulieres, I., Hubert, B., & Burack, J. (2006). Enhanced perceptual functioning in autism: an update, and eight principles of autistic perception. *J Autism Dev Disord, 36*(1), 27-43.

Mundy, P., & Sigman, M. (1989). Specifying the nature of the social impairment in autism. In G. Dawson (Ed.), *Autism: New perspectives on diagnosis, nature,*

and treatment (pp. 3-21). New York: The Guilford Press.

Mundy, P., Sigman, M., Ungerer, J., & Sherman, T. (1986). Defining the social deficits of autism: the contribution of non-verbal communication measures. *J Child Psychol Psychiatry, 27*(5), 657-669.

Munson, J., Dawson, G., Abbott, R., Faja, S., Webb, S. J., Friedman, S. D., et al. (2006). Amygdalar volume and behavioral development in autism. *Arch Gen Psychiatry, 63*(6), 686-693.

Murphy, D. G., Beecham, J., Craig, M., & Ecker, C. (2011). Autism in adults. New biologicial findings and their translational implications to the cost of clinical services. *Brain Res, 1380*, 22-33.

Nagamani, M., McDonough, P. G., Ellegood, J. O., & Mahesh, V. B. (1979). Maternal and amniotic fluid steroids throughout human pregnancy. *Am J Obstet Gynecol, 134*(6), 674-680.

Nayate, A., Bradshaw, J. L., & Rinehart, N. J. (2005). Autism and Asperger's disorder: are they movement disorders involving the cerebellum and/or basal ganglia? *Brain Res Bull, 67*(4), 327-334.

Neeley, E. S., Bigler, E. D., Krasny, L., Ozonoff, S., McMahon, W., & Lainhart, J. E. (2007). Quantitative temporal lobe differences: autism distinguished from controls using classification and regression tree analysis. *Brain Dev, 29*(7), 389-399.

Neumann, D., Spezio, M. L., Piven, J., & Adolphs, R. (2006). Looking you in the mouth: abnormal gaze in autism resulting from impaired top-down modulation of visual attention. *Soc Cogn Affect Neurosci, 1*(3), 194-202.

Newschaffer, C. J., Croen, L. A., Daniels, J., Giarelli, E., Grether, J. K., Levy, S. E., et al. (2007). The epidemiology of autism spectrum disorders. *Annu Rev Public Health, 28*, 235-258.

Nicholson, K. G., & Kimura, D. (1996). Sex differences for speech and manual skill. *Percept Mot Skills, 82*(1), 3-13.

Noipayak, P. (2009). The ratio of 2nd and 4th digit length in autistic children. *J Med Assoc Thai, 92*(8), 1040-1045.

Nopoulos, P., Flaum, M., O'Leary, D., & Andreasen, N. C. (2000). Sexual dimorphism in the human brain: evaluation of tissue volume, tissue composition and surface anatomy using magnetic resonance imaging. *Psychiatry Res, 98*(1), 1-13.

Nordahl, C. W., Dierker, D., Mostafavi, I., Schumann, C. M., Rivera, S. M., Amaral, D. G., et al. (2007). Cortical folding abnormalities in autism revealed by surface-based morphometry. *J Neurosci, 27*(43), 11725-11735.

O'Brien, L. M., Ziegler, D. A., Deutsch, C. K., Kennedy, D. N., Goldstein, J. M., Seidman, L. J., et al. (2006). Adjustment for whole brain and cranial size in volumetric brain studies: a review of common adjustment factors and statistical methods. *Harv Rev Psychiatry, 14*(3), 141-151.

O'Hearn, K., Schroer, E., Minshew, N., & Luna, B. (2010). Lack of developmental improvement on a face memory task during adolescence in autism. *Neuropsychologia, 48*(13), 3955-3960.

Oberman, L. M., & Ramachandran, V. S. (2007). The simulating social mind: the role of the mirror neuron system and simulation in the social and communicative deficits of autism spectrum disorders. *Psychol Bull, 133*(2), 310-327.

Ochsner, K. N., Ray, R. R., Hughes, B., McRae, K., Cooper, J. C., Weber, J., et al. (2009). Bottom-up and top-down processes in emotion generation: common and distinct neural mechanisms. *Psychol Sci, 20*(11), 1322-1331.

Ohnishi, T., Matsuda, H., Hashimoto, T., Kunihiro, T., Nishikawa, M., Uema, T., et al. (2000). Abnormal regional cerebral blood flow in childhood autism. *Brain, 123 (Pt 9)*, 1838-1844.

Ozonoff, S. (1997). Components of executive function in autism and other disorders. In J. Russell (Ed.), *Autism as an executive disorder* (pp. 179-211). New York: Oxford University Press.

Ozonoff, S., Iosif, A. M., Baguio, F., Cook, I. C., Hill, M. M., Hutman, T., et al. (2010). A prospective study of the emergence of early behavioral signs of autism. *J Am Acad Child Adolesc Psychiatry, 49*(3), 256-266e252.

Ozonoff, S., Pennington, B. F., & Rogers, S. J. (1991). Executive function deficits in high-functioning autistic individuals: relationship to theory of mind. *J Child Psychol Psychiatry, 32*(7), 1081-1105.

Ozonoff, S., Rogers, S. J., & Pennington, B. F. (1991). Asperger's syndrome: evidence of an empirical distinction from high-functioning autism. *J Child Psychol Psychiatry, 32*(7), 1107-1122.

Ozonoff, S., & Strayer, D. L. (1997). Inhibitory function in nonretarded children with autism. *J Autism Dev Disord, 27*(1), 59-77.

Paakki, J. J., Rahko, J., Long, X., Moilanen, I., Tervonen, O., Nikkinen, J., et al. (2010). Alterations in regional homogeneity of resting-state brain activity in autism spectrum disorders. *Brain Res, 1321*, 169-179.

Pan, C. Y., Tsai, C. L., & Chu, C. H. (2009). Fundamental movement skills in children diagnosed with autism spectrum disorders and attention deficit hyperactivity disorder. *J Autism Dev Disord, 39*(12), 1694-1705.

Panizzon, M. S., Fennema-Notestine, C., Eyler, L. T., Jernigan, T. L., Prom-Wormley,

E., Neale, M., et al. (2009). Distinct genetic influences on cortical surface area and cortical thickness. *Cereb Cortex, 19*(11), 2728-2735.

Parke, R., & Gauvain, M. (2008). *Child psychology: A contemporary viewpoint* (7th ed.). Columbus, OH: McGraw-Hill Higher Education.

Parr, J. R., Le Couteur, A., Baird, G., Rutter, M., Pickles, A., Fombonne, E., et al. (2011). Early developmental regression in autism spectrum disorder: evidence from an international multiplex sample. *J Autism Dev Disord, 41*(3), 332-340.

Patterson, K., Nestor, P. J., & Rogers, T. T. (2007). Where do you know what you know? The representation of semantic knowledge in the human brain. *Nat Rev Neurosci, 8*(12), 976-987.

Pelphrey, K. A., & Carter, E. J. (2008). Brain mechanisms for social perception: lessons from autism and typical development. *Ann N Y Acad Sci, 1145*, 283-299.

Pereira, F., Mitchell, T., & Botvinick, M. (2009). Machine learning classifiers and fMRI: a tutorial overview. *Neuroimage, 45*(1 Suppl), S199-209.

Perrin, J. S., Herve, P. Y., Leonard, G., Perron, M., Pike, G. B., Pitiot, A., et al. (2008). Growth of white matter in the adolescent brain: role of testosterone and androgen receptor. *J Neurosci, 28*(38), 9519-9524.

Pfaff, D., & Keiner, M. (1973). Atlas of estradiol-concentrating cells in the central nervous system of the female rat. *J Comp Neurol, 151*(2), 121-158.

Pickles, A., Simonoff, E., Conti-Ramsden, G., Falcaro, M., Simkin, Z., Charman, T., et al. (2009). Loss of language in early development of autism and specific language impairment. *J Child Psychol Psychiatry, 50*(7), 843-852.

Pickles, A., Starr, E., Kazak, S., Bolton, P., Papanikolaou, K., Bailey, A., et al. (2000). Variable expression of the autism broader phenotype: findings from extended pedigrees. *J Child Psychol Psychiatry, 41*(4), 491-502.

Pilowsky, T., Yirmiya, N., Shulman, C., & Dover, R. (1998). The Autism Diagnostic Interview-Revised and the Childhood Autism Rating Scale: differences between diagnostic systems and comparison between genders. *J Autism Dev Disord, 28*(2), 143-151.

Pinto, D., Pagnamenta, A. T., Klei, L., Anney, R., Merico, D., Regan, R., et al. (2010). Functional impact of global rare copy number variation in autism spectrum disorders. *Nature, 466*(7304), 368-372.

Plaisted, K., O'Riordan, M., & Baron-Cohen, S. (1998a). Enhanced discrimination of novel, highly similar stimuli by adults with autism during a perceptual learning task. *J Child Psychol Psychiatry, 39*(5), 765-775.

Plaisted, K., O'Riordan, M., & Baron-Cohen, S. (1998b). Enhanced visual search for a

conjunctive target in autism: a research note. *J Child Psychol Psychiatry, 39*(5), 777-783.

Plaisted, K., Swettenham, J., & Rees, L. (1999). Children with autism show local precedence in a divided attention task and global precedence in a selective attention task. *J Child Psychol Psychiatry, 40*(5), 733-742.

Polli, F. E., Barton, J. J., Cain, M. S., Thakkar, K. N., Rauch, S. L., & Manoach, D. S. (2005). Rostral and dorsal anterior cingulate cortex make dissociable contributions during antisaccade error commission. *Proc Natl Acad Sci U S A, 102*(43), 15700-15705.

Posserud, M. B., Lundervold, A. J., & Gillberg, C. (2006). Autistic features in a total population of 7-9-year-old children assessed by the ASSQ (Autism Spectrum Screening Questionnaire). *J Child Psychol Psychiatry, 47*(2), 167-175.

Prior, M. R. (1979). Cognitive abilities and disabilities in infantile autism: a review. *J Abnorm Child Psychol, 7*(4), 357-380.

Provost, B., Lopez, B. R., & Heimerl, S. (2007). A comparison of motor delays in young children: autism spectrum disorder, developmental delay, and developmental concerns. *J Autism Dev Disord, 37*(2), 321-328.

Radua, J., Via, E., Catani, M., & Mataix-Cols, D. (2011). Voxel-based meta-analysis of regional white-matter volume differences in autism spectrum disorder versus healthy controls. *Psychol Med, 41*(7), 1539-1550.

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proc Natl Acad Sci U S A, 98*(2), 676-682.

Rakic, P. (1988). Defects of neuronal migration and the pathogenesis of cortical malformations. *Prog Brain Res, 73*, 15-37.

Rakic, P. (1995). A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci, 18*(9), 383-388.

Rasouli, G., Rasouli, M., Lenz, F. A., Verhagen, L., Borrett, D. S., & Kwan, H. C. (2006). Fractal characteristics of human Parkinsonian neuronal spike trains. *Neuroscience, 139*(3), 1153-1158.

Raznahan, A., Lee, Y., Stidd, R., Long, R., Greenstein, D., Clasen, L., et al. (2010). Longitudinally mapping the influence of sex and androgen signaling on the dynamics of human cortical maturation in adolescence. *Proc Natl Acad Sci U S A, 107*(39), 16988-16993.

Raznahan, A., Toro, R., Daly, E., Robertson, D., Murphy, C., Deeley, Q., et al. (2010). Cortical anatomy in autism spectrum disorder: an in vivo MRI study on the effect of age. *Cereb Cortex, 20*(6), 1332-1340.

Reich, R., Cloninger, C. R., & Guze, S. B. (1975). The multifactorial model of disease transmission: I. Description of the model and its use in psychiatry. *Br J Psychiatry, 127*, 1-10.

Rilling, J. K., Barks, S. K., Parr, L. A., Preuss, T. M., Faber, T. L., Pagnoni, G., et al. (2007). A comparison of resting-state brain activity in humans and chimpanzees. *Proc Natl Acad Sci U S A, 104*(43), 17146-17151.

Ring, H., Woodbury-Smith, M., Watson, P., Wheelwright, S., & Baron-Cohen, S. (2008). Clinical heterogeneity among people with high functioning autism spectrum conditions: evidence favouring a continuous severity gradient. *Behav Brain Funct, 4*, 11.

Ritvo, E. R. (1977). Biochemical studies of children with the syndromes of autism, childhood schizophrenia and related developmental disabilities: a review. *J Child Psychol Psychiatry, 18*(4), 373-379.

Ritvo, E. R., Cantwell, D., Johnson, E., Clements, M., Benbrook, F., Slagle, S., et al. (1971). Social class factor in autism. *J Autism Child Schizophr, 1*(3), 297-310.

Rivet, T. T., & Matson, J. L. (2011). Review of gender differences in core symptomatology in autism spectrum disorders. *Research in Autism Spectrum Disorders, 5*(3), 957-976.

Robinson, S. J., & Manning, J. T. (2000). The ratio of 2nd to 4th digit length and male homosexuality. *Evol Hum Behav, 21*(5), 333-345.

Rogers, S. J., & Pennington, B. F. (1991). A theoretical approach to the deficits in infantile autism. *Development and Psychopathology, 3*(2), 137-162.

Rojas, D. C., Peterson, E., Winterrowd, E., Reite, M. L., Rogers, S. J., & Tregellas, J. R. (2006). Regional gray matter volumetric changes in autism associated with social and repetitive behavior symptoms. *BMC Psychiatry, 6*, 56.

Ronald, A., Happe, F., Bolton, P., Butcher, L. M., Price, T. S., Wheelwright, S., et al. (2006). Genetic heterogeneity between the three components of the autism spectrum: a twin study. *J Am Acad Child Adolesc Psychiatry, 45*(6), 691-699.

Ronald, A., Happe, F., Price, T. S., Baron-Cohen, S., & Plomin, R. (2006). Phenotypic and genetic overlap between autistic traits at the extremes of the general population. *J Am Acad Child Adolesc Psychiatry, 45*(10), 1206-1214.

Ropar, D., & Mitchell, P. (2001). Susceptibility to illusions and performance on visuospatial tasks in individuals with autism. *J Child Psychol Psychiatry, 42*(4), 539-549.

Rubenstein, J. L., & Merzenich, M. M. (2003). Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav, 2*(5), 255-267.

Rubinov, M., Knock, S. A., Stam, C. J., Micheloyannis, S., Harris, A. W., Williams, L.

M., et al. (2009). Small-world properties of nonlinear brain activity in schizophrenia. *Hum Brain Mapp, 30*(2), 403-416.

Ruby, P., & Decety, J. (2001). Effect of subjective perspective taking during simulation of action: a PET investigation of agency. *Nat Neurosci, 4*(5), 546-550.

Rumsey, J. M. (1985). Conceptual problem-solving in highly verbal, nonretarded autistic men. *J Autism Dev Disord, 15*(1), 23-36.

Rumsey, J. M., & Hamburger, S. D. (1988). Neuropsychological findings in high-functioning men with infantile autism, residual state. *J Clin Exp Neuropsychol, 10*(2), 201-221.

Russell, A. J., Mataix-Cols, D., Anson, M., & Murphy, D. G. (2005). Obsessions and compulsions in Asperger syndrome and high-functioning autism. *Br J Psychiatry, 186*, 525-528.

Ruta, L., Ingudomnukul, E., Taylor, K., Chakrabarti, B., & Baron-Cohen, S. (2011). Increased serum androstenedione in adults with autism spectrum conditions. *Psychoneuroendocrinology, 36*(8), 1154-1163.

Ruta, L., Mugno, D., D'Arrigo, V. G., Vitiello, B., & Mazzone, L. (2010). Obsessive-compulsive traits in children and adolescents with Asperger syndrome. *Eur Child Adolesc Psychiatry, 19*(1), 17-24.

Rutherford, M. D., & Towns, A. M. (2008). Scan path differences and similarities during emotion perception in those with and without autism spectrum disorders. *J Autism Dev Disord, 38*(7), 1371-1381.

Rutter, M. (1968). Concepts of autism: a review of research. *J Child Psychol Psychiatry, 9*(1), 1-25.

Rutter, M. (1978). Diagnosis and definition of childhood autism. *J Autism Child Schizophr, 8*(2), 139-161.

Rutter, M., Caspi, A., & Moffitt, T. E. (2003). Using sex differences in psychopathology to study causal mechanisms: unifying issues and research strategies. *J Child Psychol Psychiatry, 44*(8), 1092-1115.

Rutter, M., & Lockyer, L. (1967). A five to fifteen year follow-up study of infantile psychosis. I. Description of sample. *Br J Psychiatry, 113*(504), 1169-1182.

Ryu, Y. H., Lee, J. D., Yoon, P. H., Kim, D. I., Lee, H. B., & Shin, Y. J. (1999). Perfusion impairments in infantile autism on technetium-99m ethyl cysteinate dimer brain single-photon emission tomography: comparison with findings on magnetic resonance imaging. *Eur J Nucl Med, 26*(3), 253-259.

Sahyoun, C., Floyer-Lea, A., Johansen-Berg, H., & Matthews, P. M. (2004). Towards an understanding of gait control: brain activation during the anticipation,

preparation and execution of foot movements. *Neuroimage, 21*(2), 568-575.

Salmond, C. H., Ashburner, J., Connelly, A., Friston, K. J., Gadian, D. G., & Vargha-Khadem, F. (2005). The role of the medial temporal lobe in autistic spectrum disorders. *Eur J Neurosci, 22*(3), 764-772.

Salmond, C. H., Vargha-Khadem, F., Gadian, D. G., de Haan, M., & Baldeweg, T. (2007). Heterogeneity in the patterns of neural abnormality in autistic spectrum disorders: evidence from ERP and MRI. *Cortex, 43*(6), 686-699.

Sasson, N. J. (2006). The development of face processing in autism. *J Autism Dev Disord, 36*(3), 381-394.

Schacter, D. L., Addis, D. R., & Buckner, R. L. (2008). Episodic simulation of future events: concepts, data, and applications. *Ann N Y Acad Sci, 1124*, 39-60.

Schanen, N. C. (2006). Epigenetics of autism spectrum disorders. *Hum Mol Genet, 15 Spec No 2*, R138-150.

Schlaepfer, T. E., Harris, G. J., Tien, A. Y., Peng, L., Lee, S., & Pearlson, G. D. (1995). Structural differences in the cerebral cortex of healthy female and male subjects: a magnetic resonance imaging study. *Psychiatry Res, 61*(3), 129-135.

Schmitz, N., Rubia, K., Daly, E., Smith, A., Williams, S., & Murphy, D. G. (2006). Neural correlates of executive function in autistic spectrum disorders. *Biol Psychiatry, 59*(1), 7-16.

Scholz, J., Klein, M. C., Behrens, T. E., & Johansen-Berg, H. (2009). Training induces changes in white-matter architecture. *Nat Neurosci, 12*(11), 1370-1371.

Schulte-Ruther, M., Markowitsch, H. J., Shah, N. J., Fink, G. R., & Piefke, M. (2008). Gender differences in brain networks supporting empathy. *Neuroimage, 42*(1), 393-403.

Schultz, R. T. (2005). Developmental deficits in social perception in autism: the role of the amygdala and fusiform face area. *Int J Dev Neurosci, 23*(2-3), 125-141.

Schultz, R. T., Gauthier, I., Klin, A., Fulbright, R. K., Anderson, A. W., Volkmar, F., et al. (2000). Abnormal ventral temporal cortical activity during face discrimination among individuals with autism and Asperger syndrome. *Arch Gen Psychiatry, 57*(4), 331-340.

Schultz, R. T., Grelotti, D. J., Klin, A., Kleinman, J., Van der Gaag, C., Marois, R., et al. (2003). The role of the fusiform face area in social cognition: implications for the pathobiology of autism. *Philos Trans R Soc Lond B Biol Sci, 358*(1430), 415-427.

Schumann, C. M., Barnes, C. C., Lord, C., & Courchesne, E. (2009). Amygdala enlargement in toddlers with autism related to severity of social and communication impairments. *Biol Psychiatry, 66*(10), 942-949.

Schumann, C. M., Bloss, C. S., Barnes, C. C., Wideman, G. M., Carper, R. A., Akshoomoff, N., et al. (2010). Longitudinal magnetic resonance imaging study of cortical development through early childhood in autism. *J Neurosci, 30*(12), 4419-4427.

Schumann, C. M., Hamstra, J., Goodlin-Jones, B. L., Lotspeich, L. J., Kwon, H., Buonocore, M. H., et al. (2004). The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *J Neurosci, 24*(28), 6392-6401.

Schwarz, E., Guest, P. C., Rahmoune, H., Wang, L., Levin, Y., Ingudomnukul, E., et al. (2010). Sex-specific serum biomarker patterns in adults with Asperger's syndrome. *Mol Psychiatry*.

Sebat, J., Lakshmi, B., Malhotra, D., Troge, J., Lese-Martin, C., Walsh, T., et al. (2007). Strong association of de novo copy number mutations with autism. *Science, 316*(5823), 445-449.

Senju, A. (2011). Spontaneous theory of mind and its absence in autism spectrum disorders. *Neuroscientist*.

Shah, A., & Frith, U. (1983). An islet of ability in autistic children: a research note. *J Child Psychol Psychiatry, 24*(4), 613-620.

Shaw, P., Kabani, N. J., Lerch, J. P., Eckstrand, K., Lenroot, R., Gogtay, N., et al. (2008). Neurodevelopmental trajectories of the human cerebral cortex. *J Neurosci, 28*(14), 3586-3594.

Shehzad, Z., Kelly, A. M., Reiss, P. T., Gee, D. G., Gotimer, K., Uddin, L. Q., et al. (2009). The resting brain: unconstrained yet reliable. *Cereb Cortex, 19*(10), 2209-2229.

Shughrue, P. J., Stumpf, W. E., MacLusky, N. J., Zielinski, J. E., & Hochberg, R. B. (1990). Developmental changes in estrogen receptors in mouse cerebral cortex between birth and postweaning: studied by autoradiography with 11 beta-methoxy-16 alpha-[125I]iodoestradiol. *Endocrinology, 126*(2), 1112-1124.

Sibug, R. M., Stumpf, W. E., Shughrue, P. J., Hochberg, R. B., & Drews, U. (1991). Distribution of estrogen target sites in the 2-day-old mouse forebrain and pituitary gland during the 'critical period' of sexual differentiation. *Brain Res Dev Brain Res, 61*(1), 11-22.

Silani, G., Bird, G., Brindley, R., Singer, T., Frith, C., & Frith, U. (2008). Levels of emotional awareness and autism: an fMRI study. *Soc Neurosci, 3*(2), 97-112.

Simone, R. (2010). *Aspergirls: Empowering females with Asperger syndrome.* London, UK: Jessica Kingsley Publishers.

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J., & Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. *Science, 303*(5661), 1157-1162.

Skudlarski, P., Jagannathan, K., Calhoun, V. D., Hampson, M., Skudlarska, B. A., & Pearlson, G. (2008). Measuring brain connectivity: diffusion tensor imaging validates resting state temporal correlations. *Neuroimage, 43*(3), 554-561.

Skuse, D. H. (2000). Imprinting, the X-chromosome, and the male brain: explaining sex differences in the liability to autism. *Pediatr Res, 47*(1), 9-16.

Skuse, D. H. (2005). X-linked genes and mental functioning. *Hum Mol Genet, 14 Spec No 1*, R27-32.

Skuse, D. H. (2006). Sexual dimorphism in cognition and behaviour: the role of X-linked genes. *European journal of endocrinology, 155*(suppl_1), S99.

Skuse, D. H. (2007). Rethinking the nature of genetic vulnerability to autistic spectrum disorders. *Trends Genet, 23*(8), 387-395.

Smith, S. M., Fox, P. T., Miller, K. L., Glahn, D. C., Fox, P. M., Mackay, C. E., et al. (2009). Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A, 106*(31), 13040-13045.

Solomon, M., Miller, M., Taylor, S. L., Hinshaw, S. P., & Carter, C. S. (2011). Autism symptoms and internalizing psychopathology in girls and boys with autism spectrum disorders. *J Autism Dev Disord*.

Sowell, E. R., Peterson, B. S., Kan, E., Woods, R. P., Yoshii, J., Bansal, R., et al. (2007). Sex differences in cortical thickness mapped in 176 healthy individuals between 7 and 87 years of age. *Cereb Cortex, 17*(7), 1550-1560.

Sowell, E. R., Thompson, P. M., Leonard, C. M., Welcome, S. E., Kan, E., & Toga, A. W. (2004). Longitudinal mapping of cortical thickness and brain growth in normal children. *J Neurosci, 24*(38), 8223-8231.

Sowell, E. R., Thompson, P. M., Tessner, K. D., & Toga, A. W. (2001). Mapping continued brain growth and gray matter density reduction in dorsal frontal cortex: Inverse relationships during postadolescent brain maturation. *J Neurosci, 21*(22), 8819-8829.

Stam, C. J., de Haan, W., Daffertshofer, A., Jones, B. F., Manshanden, I., van Cappellen van Walsum, A. M., et al. (2009). Graph theoretical analysis of magnetoencephalographic functional connectivity in Alzheimer's disease. *Brain, 132*(Pt 1), 213-224.

Stanfield, A. C., McIntosh, A. M., Spencer, M. D., Philip, R., Gaur, S., & Lawrie, S. M. (2008). Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. *Eur*

Psychiatry, 23(4), 289-299.

Straube, T., Schmidt, S., Weiss, T., Mentzel, H. J., & Miltner, W. H. (2009). Sex differences in brain activation to anticipated and experienced pain in the medial prefrontal cortex. *Hum Brain Mapp, 30*(2), 689-698.

Strogatz, S. H. (2001). Exploring complex networks. *Nature, 410*(6825), 268-276.

Suckling, J. (2011). Correlated covariates in ANCOVA cannot adjust for pre-existing differences between groups. *Schizophr Res, 126*(1-3), 310-311.

Suckling, J., Barnes, A., Job, D., Brenan, D., Lymer, K., Dazzan, P., et al. (2010). Power calculations for multicenter imaging studies controlled by the false discovery rate. *Hum Brain Mapp, 31*(8), 1183-1195.

Suckling, J., & Bullmore, E. (2004). Permutation tests for factorially designed neuroimaging experiments. *Hum Brain Mapp, 22*(3), 193-205.

Suckling, J., Davis, M. H., Ooi, C., Wink, A. M., Fadili, J., Salvador, R., et al. (2006). Permutation testing of orthogonal factorial effects in a language-processing experiment using fMRI. *Hum Brain Mapp, 27*(5), 425-433.

Sullivan, S. D., & Moenter, S. M. (2004). Prenatal androgens alter GABAergic drive to gonadotropin-releasing hormone neurons: implications for a common fertility disorder. *Proc Natl Acad Sci U S A, 101*(18), 7129-7134.

Sutherland, A., & Crewther, D. P. (2010). Magnocellular visual evoked potential delay with high autism spectrum quotient yields a neural mechanism for altered perception. *Brain, 133*(Pt 7), 2089-2097.

Szatmari, P., MacLean, J. E., Jones, M. B., Bryson, S. E., Zwaigenbaum, L., Bartolucci, G., et al. (2000). The familial aggregation of the lesser variant in biological and nonbiological relatives of PDD probands: a family history study. *J Child Psychol Psychiatry, 41*(5), 579-586.

Tantam, D. (2000). Psychological disorder in adolescents and adults with Asperger syndrome. *Autism, 4*(1), 47-62.

Tantam, D. (2003). The challenge of adolescents and adults with Asperger syndrome. *Child Adolesc Psychiatric Clin N Am, 12*, 143-163.

Tantam, D. (2009). *Can the world afford autistic spectrum disorder?* London, UK: Jessica Kingsley Publishers.

Taylor, D. C., & Ounsted, C. (1972). The nature of gender differences explored through ontogenetic analyses of sex ratios in disease. In C. Ounsted & D. C. Taylor (Eds.), *Gender differences: Their ontogeny and significance.* London, UK: Churchill Livingstone.

Taylor, S. F., Martis, B., Fitzgerald, K. D., Welsh, R. C., Abelson, J. L., Liberzon, I., et al. (2006). Medial frontal cortex activity and loss-related responses to errors. *J*

Neurosci, 26(15), 4063-4070.

Teitelbaum, P., Teitelbaum, O., Nye, J., Fryman, J., & Maurer, R. G. (1998). Movement analysis in infancy may be useful for early diagnosis of autism. *Proc Natl Acad Sci U S A, 95*(23), 13982-13987.

Terrazas, A., & McNaughton, B. L. (2000). Brain growth and the cognitive map. *Proc Natl Acad Sci U S A, 97*(9), 4414-4416.

Thakkar, K. N., Polli, F. E., Joseph, R. M., Tuch, D. S., Hadjikhani, N., Barton, J. J., et al. (2008). Response monitoring, repetitive behaviour and anterior cingulate abnormalities in autism spectrum disorders (ASD). *Brain, 131*(Pt 9), 2464-2478.

Thiebaut de Schotten, M., Ffytche, D. H., Bizzi, A., Dell'Acqua, F., Allin, M., Walshe, M., et al. (2011). Atlasing location, asymmetry and inter-subject variability of white matter tracts in the human brain with MR diffusion tractography. *Neuroimage, 54*(1), 49-59.

Tiemeier, H., Lenroot, R. K., Greenstein, D. K., Tran, L., Pierson, R., & Giedd, J. N. (2010). Cerebellum development during childhood and adolescence: a longitudinal morphometric MRI study. *Neuroimage, 49*(1), 63-70.

Tiffin, J., & Asher, E. J. (1948). The Purdue pegboard; norms and studies of reliability and validity. *J Appl Psychol, 32*(3), 234-247.

Toal, F., Daly, E. M., Page, L., Deeley, Q., Hallahan, B., Bloemen, O., et al. (2010). Clinical and anatomical heterogeneity in autistic spectrum disorder: a structural MRI study. *Psychol Med, 40*(7), 1171-1181.

Tomasi, D., Chang, L., Caparelli, E. C., & Ernst, T. (2008). Sex differences in sensory gating of the thalamus during auditory interference of visual attention tasks. *Neuroscience, 151*(4), 1006-1015.

Tracy, J. L., Robins, R. W., Schriber, R. A., & Solomon, M. (2011). Is emotion recognition impaired in individuals with autism spectrum disorders? *J Autism Dev Disord, 41*(1), 102-109.

Trevarthen, C., & Aitken, K. J. (2001). Infant intersubjectivity: research, theory, and clinical applications. *J Child Psychol Psychiatry, 42*(1), 3-48.

Tsai, L. Y., & Beisler, J. M. (1983). The development of sex differences in infantile autism. *Br J Psychiatry, 142*, 373-378.

Tsai, L. Y., Stewart, M. A., & August, G. (1981). Implication of sex differences in the familial transmission of infantile autism. *J Autism Dev Disord, 11*(2), 165-173.

Tsakanikos, E., Underwood, L., Kravariti, E., Bouras, N., & McCarthy, J. (2011). Gender differences in co-morbid psychopathology and clinical management in adults with autism spectrum disorders. *Research in Autism Spectrum*

Disorders, 5, 803-808.

Turner, M. A. (1999). Generating novel ideas: fluency performance in high-functioning and learning disabled individuals with autism. *J Child Psychol Psychiatry, 40*(2), 189-201.

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage, 15*(1), 273-289.

Uddin, L. Q., & Menon, V. (2009). The anterior insula in autism: under-connected and under-examined. *Neurosci Biobehav Rev, 33*(8), 1198-1203.

Uhlhaas, P. J., & Singer, W. (2006). Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. *Neuron, 52*(1), 155-168.

Unger, R. K. (1979). Toward a redefinition of sex and gender. *American Psychologist, 34*(11), 1085-1094.

Valla, J. M., & Ceci, S. J. (2011). Can sex differences in science be tied to the long reach of prenatal hormones? Brain organization theory, digit ratio (2D/4D), and sex differences in preferences and cognition. *Perspectives on Psychological Science, 6*(2), 134-146.

van den Heuvel, M. P., Mandl, R. C., Kahn, R. S., & Hulshoff Pol, H. E. (2009). Functionally linked resting-state networks reflect the underlying structural connectivity architecture of the human brain. *Hum Brain Mapp, 30*(10), 3127-3141.

Via, E., Radua, J., Cardoner, N., Happe, F., & Mataix-Cols, D. (2011). Meta-analysis of gray matter abnormalities in autism spectrum disorder: should Asperger disorder be subsumed under a broader umbrella of autistic spectrum disorder? *Arch Gen Psychiatry, 68*(4), 409-418.

Vincent, J. L., Patel, G. H., Fox, M. D., Snyder, A. Z., Baker, J. T., Van Essen, D. C., et al. (2007). Intrinsic functional architecture in the anaesthetized monkey brain. *Nature, 447*(7140), 83-86.

Vismara, L. A., & Rogers, S. J. (2010). Behavioral treatments in autism spectrum disorder: what do we know? *Annu Rev Clin Psychol, 6*, 447-468.

Voineagu, I., Wang, X., Johnston, P., Lowe, J. K., Tian, Y., Horvath, S., et al. (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature, 474*(7351), 380-384.

Volkmar, F. R., Bregman, J., Cohen, D. J., & Cicchetti, D. V. (1988). DSM-III and DSM-III-R diagnoses of autism. *Am J Psychiatry, 145*(11), 1404-1408.

Voyer, D., Voyer, S., & Bryden, M. P. (1995). Magnitude of sex differences in spatial

abilities: a meta-analysis and consideration of critical variables. *Psychol Bull, 117*(2), 250-270.

Wada, J. A., Clarke, R., & Hamm, A. (1975). Cerebral hemispheric asymmetry in humans. Cortical speech zones in 100 adults and 100 infant brains. *Arch Neurol, 32*(4), 239-246.

Wager, T. D., Barrett, L. F., Bliss-Moreau, E., Lindquist, K. A., Duncan, S., Kober, H., et al. (2008). The neuroimaging of emotion. In M. Lewis, J. M. Haviland-Jones & L. F. Barrett (Eds.), *Handbook of Emotions* (3rd edition ed., pp. 249-271). New York, NY: Guilford Press.

Waiter, G. D., Williams, J. H., Murray, A. D., Gilchrist, A., Perrett, D. I., & Whiten, A. (2004). A voxel-based investigation of brain structure in male adolescents with autistic spectrum disorder. *Neuroimage, 22*(2), 619-625.

Waiter, G. D., Williams, J. H., Murray, A. D., Gilchrist, A., Perrett, D. I., & Whiten, A. (2005). Structural white matter deficits in high-functioning individuals with autistic spectrum disorder: a voxel-based investigation. *Neuroimage, 24*(2), 455-461.

Wallace, G. L., Case, L. K., Harms, M. B., Silvers, J. A., Kenworthy, L., & Martin, A. (2011). Diminished sensitivity to sad facial expressions in high functioning autism spectrum disorders is associated with symptomatology and adaptive functioning. *J Autism Dev Disord*.

Wang, A. T., Lee, S. S., Sigman, M., & Dapretto, M. (2007). Reading affect in the face and voice: neural correlates of interpreting communicative intent in children and adolescents with autism spectrum disorders. *Arch Gen Psychiatry, 64*(6), 698-708.

Webb, S. J., Sparks, B. F., Friedman, S. D., Shaw, D. W., Giedd, J., Dawson, G., et al. (2009). Cerebellar vermal volumes and behavioral correlates in children with autism spectrum disorder. *Psychiatry Res, 172*(1), 61-67.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence (WASI)*. New York, NY: The Psychological Corporation.

Weng, S. J., Wiggins, J. L., Peltier, S. J., Carrasco, M., Risi, S., Lord, C., et al. (2010). Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. *Brain Res, 1313*, 202-214.

Werner, E., & Dawson, G. (2005). Validation of the phenomenon of autistic regression using home videotapes. *Arch Gen Psychiatry, 62*(8), 889-895.

Wheelwright, S., Baron-Cohen, S., Goldenfeld, N., Delaney, J., Fine, D., Smith, R., et al. (2006). Predicting Autism Spectrum Quotient (AQ) from the Systemizing Quotient-Revised (SQ-R) and Empathy Quotient (EQ). *Brain Res, 1079*(1),

47-56.

White, S. J., & Saldana, D. (2011). Performance of children with autism on the Embedded Figures Test: a closer look at a popular task. *J Autism Dev Disord*.

Wicker, B., Keysers, C., Plailly, J., Royet, J. P., Gallese, V., & Rizzolatti, G. (2003). Both of us disgusted in My insula: the common neural basis of seeing and feeling disgust. *Neuron, 40*(3), 655-664.

Wilke, M., Krageloh-Mann, I., & Holland, S. K. (2007). Global and local development of gray and white matter volume in normal children and adolescents. *Exp Brain Res, 178*(3), 296-307.

Willey, L. H. (1999). *Pretending to be normal: Living with Asperger's syndrome*. London, UK: Jessica Kingsley Publishers.

Williams, J. G., Allison, C., Scott, F. J., Bolton, P. F., Baron-Cohen, S., Matthews, F. E., et al. (2008). The Childhood Autism Spectrum Test (CAST): sex differences. *J Autism Dev Disord, 38*(9), 1731-1739.

Williams, J. H., Waiter, G. D., Gilchrist, A., Perrett, D. I., Murray, A. D., & Whiten, A. (2006). Neural mechanisms of imitation and 'mirror neuron' functioning in autistic spectrum disorder. *Neuropsychologia, 44*(4), 610-621.

Wilson, L. B., Tregellas, J. R., Hagerman, R. J., Rogers, S. J., & Rojas, D. C. (2009). A voxel-based morphometry comparison of regional gray matter between fragile X syndrome and autism. *Psychiatry Res, 174*(2), 138-145.

Wing, L. (1975 / 1996). *The autistic spectrum: A guide for parents and professionals*. London, UK: Constable & Robinson Ltd.

Wing, L. (1981). Sex ratios in early childhood autism and related conditions. *Psychiatry Res, 5*(2), 129-137.

Wing, L., & Gould, J. (1979). Severe impairments of social interaction and associated abnormalities in children: epidemiology and classification. *J Autism Dev Disord, 9*(1), 11-29.

Wing, L., Gould, J., & Gillberg, C. (2011). Autism spectrum disorders in the DSM-V: better or worse than the DSM-IV? *Res Dev Disabil, 32*(2), 768-773.

Wink, A. M., Bernard, F., Salvador, R., Bullmore, E., & Suckling, J. (2006). Age and cholinergic effects on hemodynamics and functional coherence of human hippocampus. *Neurobiol Aging, 27*(10), 1395-1404.

Wink, A. M., Bullmore, E., Barnes, A., Bernard, F., & Suckling, J. (2008). Monofractal and multifractal dynamics of low frequency endogenous brain oscillations in functional MRI. *Hum Brain Mapp, 29*(7), 791-801.

Winkler, A. M., Kochunov, P., Blangero, J., Almasy, L., Zilles, K., Fox, P. T., et al. (2010). Cortical thickness or grey matter volume? The importance of selecting

the phenotype for imaging genetics studies. *Neuroimage, 53*(3), 1135-1146.

Witelson, S. F., & Kigar, D. L. (1992). Sylvian fissure morphology and asymmetry in men and women: bilateral differences in relation to handedness in men. *J Comp Neurol, 323*(3), 326-340.

Witkin, H., Oltman, P., Raskin, E., & Karp, S. (1971). *A manual for the Embedded Figures Test.* Mountain View, CA: Consulting Psychologists Press, Inc.

Witwer, A. N., & Lecavalier, L. (2008). Examining the validity of autism spectrum disorder subtypes. *J Autism Dev Disord, 38*(9), 1611-1624.

Wolff, S., & McGuire, R. J. (1995). Schizoid personality in girls: a follow-up study--what are the links with Asperger's syndrome? *J Child Psychol Psychiatry, 36*(5), 793-817.

World Health Organization. (1992). *The ICD-10 classification of mental and behavioural disorders: Clinical descriptions and diagnostic guidelines*. Geneva: World Health Organization.

Yamasue, H., Abe, O., Suga, M., Yamada, H., Rogers, M. A., Aoki, S., et al. (2008). Sex-linked neuroanatomical basis of human altruistic cooperativeness. *Cereb Cortex, 18*(10), 2331-2340.

Zahn-Waxler, C., Shirtcliff, E. A., & Marceau, K. (2008). Disorders of childhood and adolescence: gender and psychopathology. *Annu Rev Clin Psychol, 4*, 275-303.

Zarahn, E., Aguirre, G. K., & D'Esposito, M. (1997). Empirical analyses of BOLD fMRI statistics. I. Spatially unsmoothed data collected under null-hypothesis conditions. *Neuroimage, 5*(3), 179-197.

Zechner, U., Wilda, M., Kehrer-Sawatzki, H., Vogel, W., Fundele, R., & Hameister, H. (2001). A high density of X-linked genes for general cognitive ability: a run-away process shaping human evolution? *Trends Genet, 17*(12), 697-701.

Zhao, X., Leotta, A., Kustanovich, V., Lajonchere, C., Geschwind, D. H., Law, K., et al. (2007). A unified genetic theory for sporadic and inherited autism. *Proc Natl Acad Sci U S A, 104*(31), 12831-12836.

Zilbovicius, M., Boddaert, N., Belin, P., Poline, J. B., Remy, P., Mangin, J. F., et al. (2000). Temporal lobe dysfunction in childhood autism: a PET study. Positron emission tomography. *Am J Psychiatry, 157*(12), 1988-1993.

Zwaigenbaum, L. (2010). Advances in the early detection of autism. *Curr Opin Neurol, 23*(2), 97-102.

Appendix

Discrete wavelet transform and image preprocessing for the estimation of *H*

Wavelet analysis for fMRI analysis can be intuitively understood as decomposing or "atomizing" the total energy (variance) of a time series by an orthonormal basis of wavelets, each weighted by a "wavelet coefficient" representing the amount of energy in the time series at a particular scale (Bullmore et al., 2004). It provides multi-resolution analysis and has natural adaptivity to local or non-stationary features of the time series within scales of the decomposition, both are well suited to the fractal nature of fMRI time series signals (Herman, Kocsis, & Eke, 2009; Mandelbrot, 1977). Moreover, the wavelet transform is a decorrelating (whitening) transform of autocorrelated data (e.g. fMRI time series) and is a useful basis for non-parametric regression in statistical analysis.

The discrete wavelet transform (DWT) applied in the present study decomposes the total energy (variance) of a time series over a hierarchy of scales; the dyadic transform requires that the time series has a length that is a power of 2. At each scale *j*, the DWT generates a set of wavelet coefficients $d_{.,j}$, and the variance of the set of wavelet coefficients provides a way to estimate the Hurst exponent, *H*, according to the following formula:

$$\log_2\left(\mathrm{Var}\left\{d_{.,j}\right\}\right) = c + (2H+1)j + \varepsilon_j.$$

Therefore, the estimate of *H* can be obtained by regression of the log-variance of

the wavelet coefficients on the different scales. Please see Figures A1 and A2 below for an illustration. Note that the least unbiased estimator of *H* is an expectation maximization algorithm (Maxim et al., 2005) and is the method used in Chapter 6.

The DWT used in the estimation of *H* requires that time series are initially a power-of-two in length. This, of course, can be achieved by truncating any time series appropriately. In making the estimate the assumption is that the signal arises from a stationary *fractional Gaussian noise* process, and thus longer time series serve to reduce the variance of the estimates. However, in some of our previous experiments (Barnes, Bullmore, & Suckling, 2009) we have observed that transitions from rest to task-activation (and reverse) induces changes in *H*. These observations were made in contiguous 128 time-point segments and represent the minimum number of time-point considered advisable due to the limited number of wavelet scales available to make the estimation.

We would also like to give a more comprehensive account of the image preprocessing steps applied in Chapter 6. Initially, correction was made for subject motion assuming the head to be a rigid body, with translations and rotations about its centre of mass. Each 3D dataset was registered to the mean, masked image with tri-cubic spline interpolation. Residual spin excitation history effects were corrected by regressing the current ($t = 1. . .T$) and lagged (t-1) first- and second-order displacements at each voxel onto the realigned time series. Changes in global grey-level scaling during image acquisition were corrected by normalisation to the mean grey-level across all voxels, in all images. The specific effects of these preprocessing steps on the estimation of *H* are discussed in (Maxim, et al., 2005). During estimation of *H*, the decorrelating properties of the wavelet transform are exploited to remove

low-frequency signal drift and high-frequency white noise by exclusion of the lowest and highest wavelet bands respectively.

Figure A1 An example resting fMRI time series

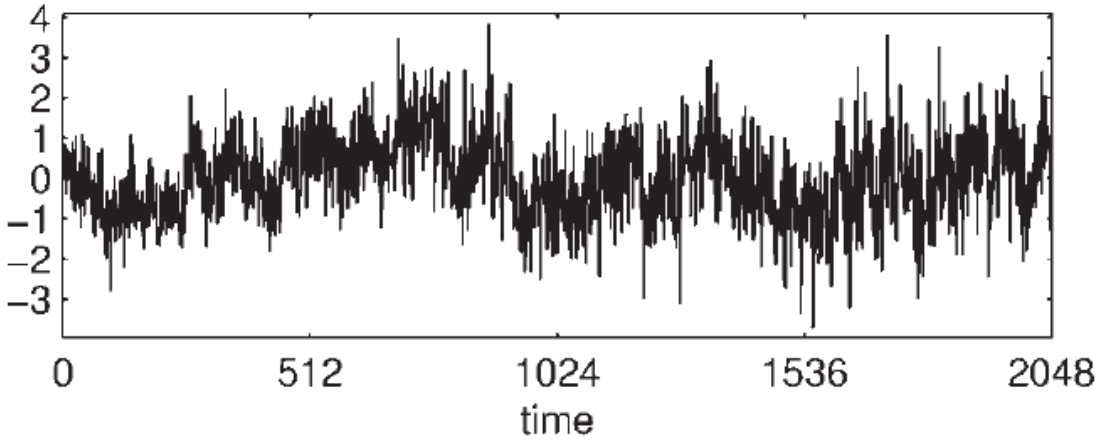
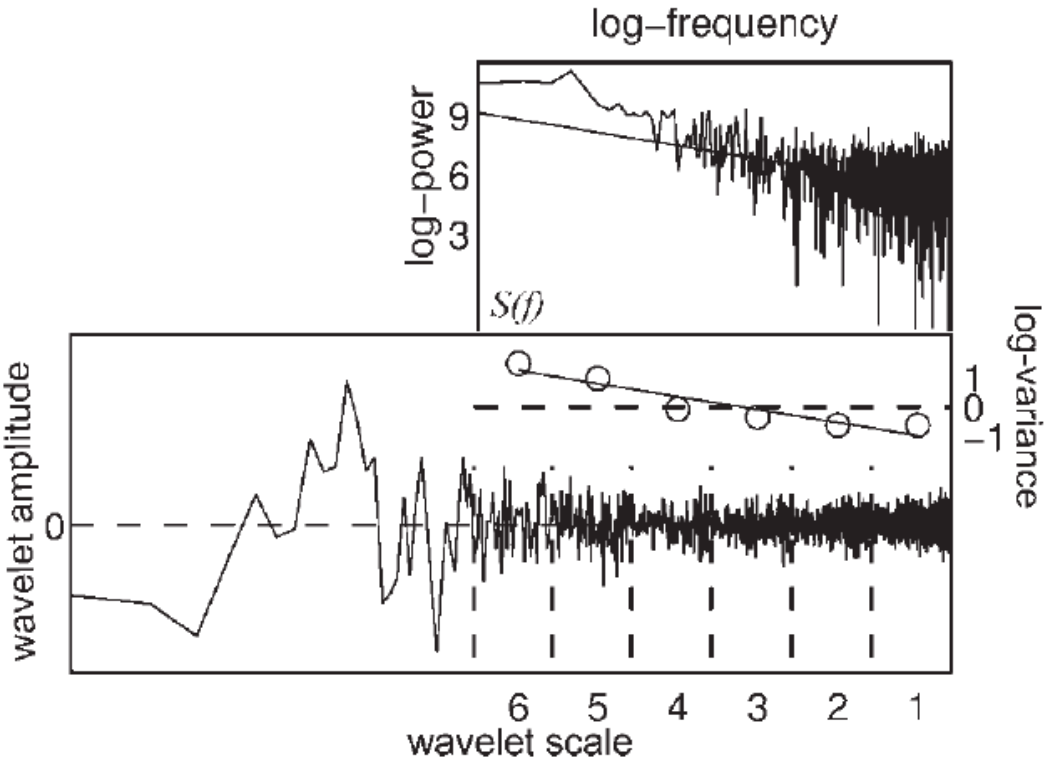


Figure A2 The power spectrum (on log-log axis) of this sample time series (upper), its wavelet transform (lower), and the log-variance at each scale (middle) of which the regression is applied to estimate the Hurst exponent of the example time series in Figure A1.



References for Appendix

Barnes, A., Bullmore, E. T., & Suckling, J. (2009). Endogenous human brain dynamics recover slowly following cognitive effort. *PLoS ONE, 4*(8), e6626.

Bullmore, E., Fadili, J., Maxim, V., Sendur, L., Whitcher, B., Suckling, J., et al. (2004). Wavelets and functional magnetic resonance imaging of the human brain. *Neuroimage, 23 Suppl 1*, S234-249.

Herman, P., Kocsis, L., & Eke, A. (2009). Fractal characterization of complexity in dynamic signals: application to cerebral hemodynamics. *Methods Mol Biol, 489*, 23-40.

Mandelbrot, B. B. (1977). *The fractal geometry of nature.* New York, NY: WH Freeman.

Maxim, V., Sendur, L., Fadili, J., Suckling, J., Gould, R., Howard, R., et al. (2005). Fractional Gaussian noise, functional MRI and Alzheimer's disease. *Neuroimage, 25*(1), 141-158.

Appendix Table 1

Within-male group differences of Hurst exponent (always lower in the ASC group) in anatomical regions previously reported as atypical in structural or perfusion neuroimaging studies of ASC[a]

Anatomical ROIs reported to be atypical in ASC brains				**Anatomical ROIs NOT reported to be atypical in ASC brains**			
	t	Sig.	ES		t	Sig.	ES
Cortical midline structures				**Primary sensory-motor cortices**			
Left superior frontal gyrus, medial	2.38	0.021 [b]	0.29	Left precentral gyrus	1.69	0.097	0.21
Right superior frontal gyrus, medial	2.05	0.045	0.25	Right precentral gyrus	1.29	0.202	0.16
Left superior frontal gyrus, medial orbital	3.37	0.001 [b]	0.40	Left postcentral gyrus	1.66	0.102	0.21
Right superior frontal gyrus, medial orbital	2.43	0.018 [b]	0.30	Right postcentral gyrus	1.22	0.227	0.15
Left anterior cingulate cortex	3.21	0.002 [b]	0.38				
Right anterior cingulate cortex	2.74	0.008 [b]	0.33	**Occipital structures**			
Left middle cingulate cortex	2.95	0.005 [b]	0.35	Left superior occipital gyrus	1.13	0.261	0.14
Right middle cingulate cortex	1.99	0.052	0.25	Right superior occipital gyrus	1.50	0.139	0.19
Left posterior cingulate cortex	3.30	0.002 [b]	0.39	Left middle occipital gyrus	1.63	0.108	0.20
Right posterior cingulate cortex	2.65	0.010 [b]	0.32	Right middle occipital gyrus	1.51	0.137	0.19
Frontal structures							
Left inferior frontal gyrus, pars opercularis	2.63	0.011 [b]	0.32				

Parietal structures			
Left angular gyrus	2.00	0.050	0.25
Right angular gyrus	1.80	0.078	0.22
Left supramarginal gyrus	2.67	0.010 [b]	0.32
Right supramarginal gyrus	2.89	0.005 [b]	0.35
Temporal-limbic structures			
Left superior temporal gyrus	2.43	0.018 [b]	0.30
Right superior temporal gyrus	2.24	0.029 [b]	0.28
Left superior temporal pole	2.68	0.009 [b]	0.32
Right superior temporal pole	2.19	0.032 [b]	0.27
Left hippocampus	3.40	0.001 [b]	0.40
Right hippocampus	2.69	0.009 [b]	0.33
Left fusiform gyrus	2.42	0.018 [b]	0.30
Right fusiform gyrus	2.21	0.031 [b]	0.27
Left insula	2.66	0.010 [b]	0.32
Right insula	2.67	0.010 [b]	0.32
Left amygdala	2.65	0.010 [b]	0.32
Right amygdala	2.78	0.007 [b]	0.33
Subcortical structures			
Left caudate nucleus	2.59	0.012 [b]	0.31
Right caudate nucleus	2.97	0.004 [b]	0.36
Left thalamus	2.73	0.008 [b]	0.33
Right thalamus	2.92	0.005 [b]	0.35

[a]: Regions defined by AAL-90 anatomical template. All statistical tests were parametric (independent sample *t* test).

[b]: $p < .032$ (corrected for multiple comparisons under false-discovery-rate $q < .05$), two-tailed.

Appendix Figure 1

Absolute difference of Hurst exponent (H) is roughly negatively correlated to wavelet-filtered low frequency (0.024-0.048 Hz) correlation coefficients for each pair of regions. Left-upper graph includes all participants into calculation; right upper includes only the male neurotypical control group and right lower only the male ASC group. Each dot represents a pair-wise relationship; there were totally 4005 data-points (i.e., all the pair-wise relationship for the 90 parcellated regions according to the AAL template on cortical and subcortical regions). The significant negative correlation shows that regions with closer H (i.e., smaller delta-H) have higher correlation coefficients, whilst regions with larger differences in H have weaker functional connectivity in between. This suggests that two regions with similar fractal scaling (thus having similar H) may have higher functional connectivity (i.e., they are more "synchronised"), and vice versa. However the shape of the distribution indicates that the relationship between differences in H and functional connectivity should not be considered a simple linear correlation. We suspect there are multiple correlation patterns, possibly depending on specific classification of regions.