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Brain Structure, Function and Connectivity in Autism Spectrum Disorders

Dr. Jane McGrath

Thesis submitted for the Degree of Doctor of Philosophy

Department of Psychiatry,

University of Dublin,

Trinity College

2012

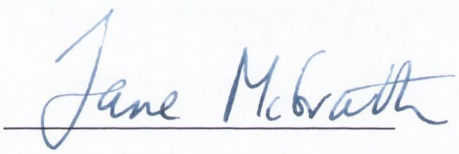
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Dr Jane McGrath, MB, MRCPsych

15th June 2012

Acknowledgements

I am deeply grateful to every person who has helped me, directly and indirectly, with this study. It is only now, at the end of weeks of scientific writing, that I sit down to say thank you and I am honestly struck by how many people have given me their time, help, knowledge, advice, support and encouragement over the past four years. Thank you all.

Firstly, I am indebted to all the families who have taken part in this study. Participation took up a significant amount of time, and some families trekked though snow and floods to come for scanning in Trinity! I really appreciate everyone's willingness and good humour throughout, especially during the scanner malfunctions and fire alarms mid-testing! Thank you all for your interest, encouragement and insight. Your involvement in autism research is greatly appreciated.

I would like to offer my sincerest gratitude to my supervisors, Professor Louise Gallagher, Dr Katherine Johnson and Professor Hugh Garavan for their support and input to this thesis. They have gone out of their way to help me time and time again, and have been a source of inspiration, wisdom and invaluable advice. I am extremely grateful to you all.

I would also like to thank everyone in the Department of Psychiatry in Trinity College, in particular Professor Michael Gill, Dr Nadia Bolshakova, Dr Emma Jane Rose, Dr Ric Anney, Dr Gary Donohue, Ms Sonja Delmonte, Ms Jackie Fitzgerald, Dr Arun Bokde, Professor Thomas Frodl, Professor Aidan Corvin, Dr Carlos Pinto, Dr Derek Morris, and Ms Geraldine Quinn. Geraldine, thank you for keeping me organized and sane! A big thanks also to Sean Brennan and Dr Miriam Law-Smith for help with data collection and also for invaluable advice on Word and EndNote!

I owe a huge thank you to a number of people for pointing me in the right direction in terms of neuroimaging methods and analytic techniques, especially to Dr Erik O'Hanlon and Professor Alexander Leemans. Erik taught me how to analyse functional MRI and diffusion MRI data and I am extremely grateful to him for his teaching, longstanding patience and good nature during the project! Alexander has also been an inspiration in relation to diffusion MRI analysis; he has been enthusiastic and insightful. I would also like to thank Dr Stephen Meredith, Dr Dara Cannon, Dr Kathleen Curran, Professor Richard Reilly, Dr Flavio Dell'Aqua, Dr Luka Pugliese, Dr Marco Catani, Professor Declan Murphy, Dr Christine Ecker and Dr Jill O'Reilly for advice about neuroimaging analysis.

Thank you to everyone in Trinity College Institute of Neuroscience. I am indebted to Dr Sojo Joseph who patiently helped me to scan every participant in this study; he has a remarkable ability to settle everyone with his calm and reassuring manner. To Adam Stone, Brendan Behan and Aisling Whitaker, thank you! When I started this study I didn't know what a command line was but thanks to you I now know how to use my fancy computer! Thanks also for talking through so much of my analysis with me. Thanks to everyone in room 3.01; Aisling, Jeni, Dan, Joanne, Sarah, Owen, Omar, Maeve and Corina, it was a pleasure to work with you all. To David, Ruth, Katie, Emma, Dave, Ian – thank you for putting up with those long noisy DTI scans while we sorted out numerous problems with the acquisition sequence! Thank you to all my friends for support, encouragement and for listening – when things were good and when things went wrong! I am very grateful to everyone in the main office who has helped me with an enormous variety of issues over the past four years; Ciaran Conneely, Lisa Beattie, Aida Gutkauskaite, Barbara Hewitt and Gillian Roddie. Thanks to Dr Colm Connolly, Dr Christian Kerskens, Dr Jason Chan and Dr Christina Simoes-Franklin who all helped in different ways to get this study off the ground! I am grateful to the Trinity Centre for High Performance Computing (TCHPC) for permission to use their computing facilities and in particular, thank you to Jimmy Tang for helping me resolve problems that I thought were insurmountable!

This project would not have been possible without funding, and I would like to sincerely thank Molecular Medicine Ireland for granting me a Clinician Scientist Fellowship and providing all funding for this work.

Finally, and most especially, I would like to thank my family. To my parents Sheila and Ian and parents-in-law Liz and Mick, thank you for all your love and encouragement (as well as the invaluable freezer food, babysitting, and house cleaning!). To Ruth, David, Orla, Lenny, No-no, Damian, Jen, Mary-Liz, Hugh and Alice, thank you to you all for your good humour, help and kindness! To my darling Ben and Abby, thank you for putting things into perspective and making everything fun! And to my husband Guy, thank you for everything - a detailed review of all you have done would probably be longer than this thesis!! There is no way that I could have completed this without you.

Jane McGrath,

University of Dublin, Trinity College,

June 2012

Related publications

Peer reviewed publications directly related to thesis

- **McGrath J**, Johnson KA, Ecker C, O'Hanlon E, Gill M, Gallagher L, Garavan H. Atypical visuospatial processing in autism; insights from functional connectivity analysis. Autism Research 2012 Oct;5(5):314-30.
- **Sanders J**, Johnson KA, Garavan H, Gill M, Gallagher L. A review of neuropsychological and neuroimaging research in autistic spectrum disorders: Attention, inhibition and cognitive flexibility. Research in Autism Spectrum Disorders 2007, Volume 2, Issue 1, January-March 2008, Pages 1-16.

Manuscripts in preparation

- Tract-Based Spatial Statistics in Autism Spectrum Disorders
- Constrained Spherical Deconvolution based tractography in Autism Spectrum Disorders
- Linking structural and functional connectivity in Autism Spectrum Disorders

Conference presentations

- **McGrath J**, Johnson KA, O'Hanlon E, Ecker C, Gallagher L, Garavan H. Neural connectivity in autism spectrum disorders – linking brain structure and brain function. Oral presentation at the Dublin Centre for Clinical Research Inaugural Scientific Meeting, Trinity College Dublin 14th October 2011.
- **McGrath J**, Johnson KA, O'Hanlon E, Ecker C, Gallagher L, Garavan H. Brain Connectivity in Autism. Oral presentation at ACAMH (Association of Child and Adolescent Mental Health) Annual Research Day, 12th October 2011.
- **McGrath J**, Johnson KA, Ecker C, Whelan R, Garavan H, Gallagher L. Brain Structure, Function and Connectivity in Autism Spectrum Conditions. Poster presentation at the American Academy of Child and Adolescent Psychiatry (AACAP) and Canadian Academy of Child and Adolescent Psychiatry (CACAP) Joint Annual Meeting, Toronto, Canada, October 2011.
- **McGrath J**, Johnson KA, O'Hanlon E, Ecker C, Gill M, Gallagher L, Garavan H. Neural connectivity in autism spectrum disorders. Oral presentation at Molecular Medicine Ireland Annual Scientific Meeting, Dublin, July 2011.

- **McGrath J**, Johnson KA, Ecker C, Gallagher L, Garavan H. Functional Connectivity During Mental Rotation in Autism. Poster presentation at Wiring the Brain International Conference, Co. Wicklow, 14-16 April 2011.
- **McGrath J**, Johnson KA, Ecker C, Garavan H, Gallagher L. Diffusion Tensor Imaging in Autism Spectrum Disorders. Poster presentation at Wiring the Brain International Conference, Co. Wicklow, 14-16 April 2011.
- **McGrath J**, Johnson KA, Garavan H, Ecker C, Gill M, Gallagher L. Brain Function and Connectivity in Autism Spectrum Disorders. IMFAR (International Meeting for Autism Research), Chicago, Illinois, USA, May 2010.

Peer reviewed publications indirectly related to thesis

- Rose EJ, Morris DW, Fahey C, Robertson IH, Greene C, O'Doherty J, Newell FN, Garavan H, **McGrath J**, Bokde Arun, Tropea D, Gill M, Corvin AP, Donohoe G. The effect of the neurogranin schizophrenia risk variant rs12807809 on brain structure and function. (accepted manuscript; Twin Research and Human Genetics: ENIGMA special issue)
- Rose EJ, Greene C, Kelly S, Morris DW, Robertson IH, Fahey C, Jacobson S, O'Doherty J, Newell FN, **McGrath J**, Bodke A, Garavan H, Frodl T, Gill M, Corvin AP, Donohoe G. The NOS1 variant rs6490121 is associated with variation in prefrontal function and grey matter density in healthy individuals. NeuroImage 2012; 60: 614-22.
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- Anney R et al. A genome-wide scan for common alleles affecting risk for autism. Human Molecular Genetics 2010. 19(20): 4072-82.
- Pinto D et al. Functional impact of global rare copy number variation in autism spectrum disorders. Nature 2010. 466(7304):368-72.

Book chapters

- Gill M, **McGrath J**. Genetics. In Psychiatry: An evidence-based text (2010). Chapter 33: 465-485. Eds: Basant Puri and Ian Treasaden.

Summary of methods used

In this study, two neuroimaging modalities were used to investigate neural connectivity in individuals with autism spectrum disorder; functional magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI).

Functional MRI (fMRI) is a neuroimaging method that measures changes in blood oxygenation in the brain, allowing for the detection of areas of the brain that are active at a particular time-point. fMRI can be used to map the neural regions that are involved in specific cognitive functions, and to assess 'functional connectivity' between brain regions. Functional connectivity is defined as the "temporal correlation between spatially remote neurophysiological events" (Friston *et al.*, 1993) and it is often interpreted as an indirect measure of the communication or co-ordination of processing between brain regions. A type of functional connectivity analysis called psychophysiological interactions analysis (PPI) was used in this study. PPI analysis allows investigation of how brain regions interact specifically during a psychological task; in this study all participants completed a mental rotation paradigm.

Diffusion tensor imaging (DTI) is an MRI method that assesses the underlying architectural organisation of white matter tracts through measurement of the restricted diffusion of water molecules in tissue (Basser and Jones, 2002; Jones and Leemans, 2011). It permits quantification of the directionality and coherence of neuronal fibres (Stieltjes *et al.*, 2001) and therefore represents an important method for measuring structural connectivity in humans (Stieltjes *et al.*, 2001; Alexander *et al.*, 2007). Two methods of analysis of DTI data were used in this study. First, Tract-Based Spatial Statistics (TBSS) (http://www.fmrib.ox.ac.uk/fsl/tbss/), an automated, observer-independent method that performs voxel-wise comparisons of diffusion properties of white matter was used to investigate whole brain white matter microstructure. Second, Constrained Spherical Deconvolution (CSD) based tractography, an analytic process that essentially uses the orientation of the diffusion profile to reconstruct the trajectories of white matter fibre bundles, was used to isolate specific white matter tracts in the brain. Importantly, this advanced CSD-based approach overcomes significant limitations associated with traditional diffusion tensor-based tractography (Tournier *et al.*, 2008).

Main findings of the thesis

In the first part of this study, functional brain activity and functional connectivity was measured during a visuospatial processing paradigm of mental rotation. ***Behavioural results*** from this study revealed a previously unreported dissociation between ASD and control groups in response times during mental rotation whereby neurotypical controls slowed significantly on the more cognitively demanding trials but the ASD group did not show this slowing. This finding may reflect a relative superiority of visuospatial processing in the ASD group. ***Functional MRI results*** indicated that in the ASD group, brain activation during mental rotation was significantly reduced in frontal, parietal, occipital, temporal and cerebellar

regions. Therefore individuals with ASD successfully performed the mental rotation task but with significantly reduced cortical involvement. Results from the ***functional connectivity analyses*** provided a novel insight into the neural underpinnings of the behavioural results, and, on a wider scale, into the neural mechanisms of atypical visuospatial processing in ASD. There was reduced functional connectivity between multiple brain regions during mental rotation in ASD, a finding in keeping with a large body of literature. Some connections appeared to be conserved, and were recruited in a qualitatively different way by the two groups; ASD and control groups show opposing directions of negative/positive functional connectivity between a number of regions during mental rotation in this study. Atypical visuospatial processing in ASD appears to be associated with both quantitative and qualitative differences in functional connectivity, which may result in a combination of enhanced low-level perceptual processing and a reduction of higher-level cortical control.

In the second part of this study, DTI data was analysed. At the whole brain level, ***TBSS analysis*** of DTI data revealed that relative to controls, the ASD group showed significant disruption of white matter microstructural organization in small regions of seven major association, projection and commissural tracts. Using ***CSD-based tractography***, six major white matter tracts were isolated (bilateral inferior fronto-occipital fasciculus (IFOF), bilateral arcuate fasciculus (AF), forceps major and forceps minor). Analysis of diffusion measures in these tracts revealed that there was abnormal microstructural organisation in the right IFOF and in the forceps major in the ASD group. Of interest, diffusion metrics in these tracts correlated with behavioural measures; increased severity of stereotyped behaviours and restricted interests correlated with reduced integrity of white matter in the forceps major, and poorer visuospatial processing was associated with disrupted white matter in the right IFOF.

The ultimate aim of this study was to investigate, for the first time, how structural connectivity, functional connectivity and behaviour are related in ASD. Despite the extensive evidence for disrupted functional and structural connectivity in ASD, no previous studies have used a ***multimodal approach to integrate functional and structural connectivity analysis.*** CSD-based tractography isolated white matter tracts that directly linked pairs of brain regions that were abnormally functionally connected. Analysis of white matter in these tracts revealed that microstructural organisation is disrupted in some, but not all, tracts that link regions that are abnormally functionally connected. In addition, there are strong correlations between measures of white matter microstructure, functional connectivity and behaviour, which provide a fascinating insight into the relationships between brain structure, brain function and information processing in both neurotypical controls and individuals with ASD. This novel multimodal imaging study reports, for the first time, direct associations between abnormal functional connectivity and disrupted white matter structure in ASD and reveals that there are significant correlations between the microstructural integrity of white matter, functional connectivity, visuospatial processing speed and autism severity.

Abbreviations

ABIDE	Autism Brain Imaging Data Exchange
AD	Axial Diffusivity
ADC	Apparent Diffusion Co-efficient
ADI-R	Autism Diagnostic Interview - Revised
ADOS	Autism Diagnostic Observation Schedule
AF	Arcuate fasciculus
ANOVA	Analysis of Variance
ASD	Autism Spectrum Disorder
ATR	Anterior thalamic radiation
BA	Brodmann Area
BOLD	Blood Oxygen Level Dependant contrast
CC	Corpus callosum
CL	Linear diffusion co-efficient
CNTNAP2	Contactin associated protein-like 2
CNV	Copy Number Variant
CP	Planar diffusion co-efficient
CS	Spherical diffusion co-efficient
CSD	Constrained Spherical Deconvolution
CST	Corticospinal tract
DTI	Diffusion Tensor Imaging
DTT	Diffusion Tensor Tractography
DSM	Diagnostic and Statistical Manual of Mental Disorders
DWI	Diffusion weighted image
DW-MRI	Diffusion weighted Magnetic Resonance Imaging
EEG	Electroencephalography
EFT	Embedded Figures Test
EPF	Enhanced Perceptual Functioning

FA	Fractional Anisotropy
FDR	False discovery rate
fMRI	Functional Magnetic Resonance Imaging
FOD	Fibre Orientation Distribution
F maj	Forceps major
FWE	Familywise Error
GABA	Gamma-aminobutyric acid
GLM	General Linear Model
HARDI	High Angular Resolution Diffusion Imaging
IFOF	Inferior fronto-occipital fasciculus
ILF	Inferior longitudinal fasciculus
ICA	Independent Component Analysis
ICD	International Classification of Diseases
IQR_L	Lower interquartile range
MD	Mean Diffusivity
MEG	Magnetoencephalography
MNI	Montreal Neurological Institute
MRI	Magnetic Resonance Imaging
MRT	Mean Response Time
N	Number of diffusion-weighting directions
NF1	Neurofibromatosis 1
NLGN3	Neuroligin 3
NLGN4X	Neuroligin 4-x linked
NPM	Non-Parametric Mapping
NRXN1	Neurexin 1
OR	Optic radiation
PET	Positron Emission Tomography
PPI	Psychophysiological interactions analysis
PTEN	Phosphatase and tensin homologue

RD	Radial Diffusivity
ReHo	Regional Homogeneity
RELN	Reelin gene
RESTORE	Robust Estimation of Tensors by Outlier Rejection
ROI	Region of interest
RT	Response Time
SEM	Structural Equation Modelling
SHANK 3	SH3 and multiple ankyrin repeat domains 3
SLF	Superior longitudinal fasciculus
SRS	Social Responsiveness Scale
TBSS	Tract based spatial statistics
TE	Echo time
TR	Repetition time
TSC 1 / 2	Tuberous sclerosis 1 and 2
UF	Uncinate fasciculus
VBM	Voxel-based morphometry
WCC	Weak Central Coherence

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1. Introduction

1. Background

1.1. History

Autism and autism spectrum disorders (ASDs) are devastating neurodevelopmental disorders of childhood with deficits in social interaction and communication and characteristic restricted, repetitive patterns of behaviours, interest and activities. One hundred years ago, a Swiss psychiatrist called Eugen Bleuler first used the term "autism" to describe a group of symptoms in patients with schizophrenia (Bleuler, 1911). Autism is a word derived from 'autos' - the Greek for 'self', and Bleuler used the term in reference to symptoms of social withdrawal or 'self-isolation' in schizophrenia. In the 1940s, Leo Kanner, a doctor from Johns Hopkins University, began using the term autism to describe a similar group of symptoms in children and at about the same time, an Austrian paediatrician, Hans Asperger, identified a similar condition in children (Kanner, 1943; Asperger, 1944).

1.2. Classification and diagnosis

Autism is classified in ICD-10 (International Classification of Diseases; World Health Organisation, 1992) and DSM-IV (Diagnostic and Statistical Manual of Mental Disorders; American Psychiatric Association, 1994) within the Pervasive Developmental Disorders. Also contained within this diagnostic grouping are atypical autism, Asperger Syndrome and Pervasive Developmental Disorder – Not Otherwise Specified. The term ASD is commonly used to describe conditions on the autistic spectrum not meeting criteria for autism as defined by ICD-10 or DSM-IV. Asperger syndrome describes individuals with core deficits in social interaction, communication and behaviour with normal intellectual functioning and no history of speech and language delay. High functioning autism commonly refers to individuals meeting criteria for autism with normal intellectual functioning and a history of speech and language delay. The diagnosis of autism and ASD is based on clinical observation of behaviour; there are no objective diagnostic tests

for autism and no biological markers have been found to reliably diagnose autism in an individual person (Ecker *et al.*, 2010).

1.3. Epidemiology

The reported prevalence of ASDs has increased over the past 20 years, with rates for childhood autism of 38.9 per 10,000, and 116.1 per 10,000 for all ASDs, from one study of 56,964 children in the South Thames region of the UK (Baird *et al.*, 2006). The combined prevalence of ASD is now approaching 1% in the human population (Gilman *et al.*, 2011). Changes in prevalence may be attributable to methodological changes in epidemiological studies, changes in diagnostic, classification and inclusion criteria, and increased awareness and education among the clinical and general communities (Kielinen *et al.*, 2000; Honda *et al.*, 2005; Chen *et al.*, 2007). The male to female ratio in ASD is approximately 4:1, although when only severe cases of autistic disorder are considered, the ratio approaches 1:1. The deficits associated with autism are enduring. Co-morbid features, such as depression, anxiety, attention deficit and hyperactivity disorder, obsessional features and oppositionality, frequently complicate the clinical picture and cause difficulties with clinical management.

1.4. Aetiology

1.4.1. Genetics

Autism has a strong genetic basis (Bailey *et al.*, 1995; Bailey *et al.*, 1996) and the estimated heritability of autism is thought to be as high as 90% (Hyman, 2008) although a recent twin study reported a much lower heritability estimate of 37% (Hallmayer *et al.*, 2011). The relative risk of a child being diagnosed with autism is increased at least 25-fold over the population prevalence in families in which a sibling is affected (Jorde *et al.*, 1991; Bolton *et al.*, 1994). First-degree relatives of a child with an ASD are more likely than controls to show subtle cognitive or behavioural features that are qualitatively similar to those observed in probands (the broader autism phenotype) (Bolton *et al.*, 1994; Bishop *et al.*, 2004). Twin studies have reported that concordance rates for monozygotic twins (70–90%) are

very significantly higher than the corresponding values for dizygotic twins (0–10%) (Steffenburg *et al.*, 1989; Bailey *et al.*, 1995)

Despite the strong genetic component, the aetiology of autism is poorly understood both at the cellular and the molecular level. Nevertheless, there are theories about abnormal neuronal connectivity and defective synaptic function, which are gaining support from recent genetic findings (e.g. (Bourgeron, 2009; Gilman *et al.*, 2011) and which may account for the core features of autism. Earlier investigations into the genetic risk factors for autism used methods such as family based linkage studies and the identification of chromosomal abnormalities. Rapid developments in technologies have led to whole genome association studies, multiple methodologies to identify structural chromosomal rearrangements or copy number variants (CNV) and more recently whole exome sequencing. A detailed description of the genetics of autism is beyond the scope of this thesis, however emerging data on the role of CNV and structural rearrangements in autism has highlighted the role of genes involved in neurodevelopment, specifically synaptogenesis, cell motility and genes within the post-synaptic density (see Geschwind *et al.*, 2011, Marshall and Scherer 2012 for recent overviews).

Recently a large biological network of genes affected by de novo CNVs in ASDs was identified through network-based analysis of genetic associations (Gilman *et al.*, 2011). Interestingly, the genes forming the network are known to be primarily involved in the development and maturation of synaptic contacts in the brain. The authors suggested that disrupted synaptogenesis may be a core pathogenic mechanism in ASDs, a finding in keeping with a large body of neuroimaging research suggesting that ASD is primarily a disease of neuronal connectivity. Genetic mutations have been identified in major molecular pathways involved in synaptic development and homeostasis (Bourgeron, 2009). Mutations identified in genes involved in the neurexin/neuroligin/SHANK pathway (neuroligin 3 (NLGN3), neuroligin 4-x linked (NLGN4X) (Jamain *et al.*, 2003), SH3 and multiple ankyrin repeat domains 3 (SHANK3) (Durand *et al.*, 2007), SHANK 2 (Leblonde *et al.*, 2012), neurexin 1 (NRXN1) (Kim *et al.*, 2008) and contactin

associated protein-like 2 (CNTNAP2) (Alarcon *et al.*, 2008; Arking *et al.*, 2008; Bakkaloglu *et al.*, 2008) are thought to disrupt synaptic function through alteration of synaptogenesis and synaptic homeostasis. The interaction between neurexin and neuroligin proteins at the synapse is thought to play an important role in controlling the balance between excitatory glutamate and inhibitory gamma-aminobutyric acid (GABA) inputs. Interestingly, a recent study reported a significant association between variation in contactin-associated protein-like 2 (CNTNAP2) a member of the neurexin gene superfamily that has been associated with autism, and a functional connectivity phenotype in the human brain (Scott-Van Zeeland *et al.*, 2010). This study provides evidence to suggest that genetic variation at CNTNAP2 predisposes to diseases such as autism in part through modulation of frontal lobe connectivity. Mutations in the tumour suppressor genes tuberous sclerosis 1 and 2 (TSC1/2), neurofibromatosis 1 (NF1) and phosphatase and tensin homologue (PTEN) genes have also shown association with autism (Baker *et al.*, 1998; Butler *et al.*, 2005) and are thought to increase activity of the mTOR/PI3K pathway, an intracellular signaling pathway which typically regulates cellular growth in mitotic cells. It is possible that mutations in this pathway lead to an excess of synaptic/cell growth and abnormal synaptic function due to an excess of protein synthesis at the synapse (Bourgeron, 2009).

1.4.2. Abnormal neural connectivity

In parallel with genetic research, neuroimaging research has demonstrated numerous abnormalities in brain structure and function in autism, from which a number of theories of altered cortical connectivity in autism have emerged (Brock *et al.*, 2002; Frith, 2003; Belmonte *et al.*, 2004; Just *et al.*, 2004; Courchesne and Pierce, 2005; Just *et al.*, 2007; Kana *et al.*, 2011). Together, these theories hold that core social and cognitive features of autism are underpinned by abnormal interregional brain connectivity. This idea is compelling as it could unify a number of the current neuropsychological theories about the aetiology of autism. For example disrupted neural connectivity in autism could contribute to restricted and repetitive behaviour (the executive dysfunction hypothesis (Turner, 1999)),

social and communication deficits (the empathising-systemising theory (Baron-Cohen, 2002)), weak central coherence (the weak central coherence theory (Frith and Happe, 1994; Happe and Frith, 2006)), and superior perceptual function (the enhanced perceptual functioning theory (Mottron *et al.*, 2006)). The *executive dysfunction hypothesis* of autism holds that deficits in executive control over behaviour account for the defining behavioural features of autism (Turner, 1999). This theory addresses the restricted, repetitive, stereotyped patterns of behaviour characteristic of autism and it holds that due to anatomical dysfunction within the fronto-striatal and fronto-parietal circuits, people with autism will demonstrate difficulties with higher-order cognitive functions, such as sustained attention, response inhibition and cognitive flexibility (Pennington and Ozonoff, 1996; Verte *et al.*, 2005). Abnormal neural connectivity between fronto-striatal and fronto-parietal regions may therefore lead to executive dysfunction. The *empathising-systematising theory* of autism outlines abnormal psychological functions that may explain the social and communication deficits characteristic of autism (Baron-Cohen, 2002). This theory postulates that ASD involves deficits in the normal development of empathy in conjunction with superior ability in 'systematising', a term referring to the drive to analyse, explore and construct systems. Abnormal neural connectivity between key 'social' brain regions may contribute to the impaired processing of social signals in individuals with ASD (von dem Hagen *et al.*, 2012). The *weak central coherence* theory suggests that there is superior local processing in ASD, possibly combined with a deficit in global integration of information (Happe and Frith, 2006). This hypothesis can account for the unusual attentional features seen in autism; for example fascination with unusual objects or unusual aspects of objects. The *enhanced perceptual functioning* theory also holds that there is superior locally oriented processing in ASD coupled with enhanced perceptual operations (Mottron *et al.*, 2006), which for example may contribute to enhanced visuospatial performance in the condition. Abnormal neural connectivity could contribute significantly to the superior locally oriented processing described in both these theories (Just *et al.*, 2004; Liu *et al.*, 2011; Wass, 2011).

2. Investigating the theory of abnormal cortical connectivity in ASD

Brain "functional connectivity" and "structural connectivity" have been investigated in ASD, and these studies have provided accumulating evidence for the theory of abnormal cortical connectivity. Functional connectivity refers to the co-ordination of processing, or communication, between brain regions and can be measured using functional magnetic resonance imaging (fMRI), Positron Emission Tomography (PET), electroencephalography (EEG), magnetoencephalography (MEG) and regional homogeneity (ReHo) analyses. Structural connectivity is a term referring to the micro- and macro-structural integrity of white matter (the nerve fibres) of the brain and can be evaluated using structural MRI, diffusion tensor imaging and post-mortem histological analysis. In this section, an overview of studies investigating both functional and structural connectivity in ASD is provided.

2.1. Functional connectivity in ASD

Functional connectivity analysis focuses on examining cortical activation at a systems level rather than at the level of local brain regions. It has become evident that successful performance of discrete cognitive functions often depends on activation in networks of interacting cortical areas - not on activation in a single brain region (e.g. (Corbetta *et al.*, 2002; Ecker *et al.*, 2006)). Functional connectivity analysis measures the level of synchronization between brain regions by computing the correlation between the brain activation levels in two activated areas over a set time period. It has been defined as the "temporal correlation between spatially remote neurophysiological events" (Friston *et al.*, 1993) and functional connectivity analysis is now widely used in fMRI studies for detection and analysis of large networks that co-activate. Functional connectivity is often interpreted as an indirect measure of the communication or co-ordination of processing between brain regions.

Functional connectivity research has revealed that there is abnormal inter- and intra-regional connectivity in ASD, which is characterised by both reduced and increased connectivity. Initially it was thought that abnormal

functional connectivity in ASD was characterised solely by reduced long-range fronto-posterior functional connectivity. More recent studies have, however, revealed that the picture is more complex; there are now reports of reduced functional connectivity between additional non fronto-posterior brain regions, of increased *inter*-regional functional connectivity and of increased *intra*-regional functional connectivity. Each of these will be described below.

2.1.1. Reduced functional connectivity in ASD

Reduced functional connectivity between frontal and posterior areas has been reported during cognitive tasks of social cognition (Mason *et al.*, 2008; Kana *et al.*, 2009), working memory (Luna *et al.*, 2002; Koshino *et al.*, 2005; Kleinhans *et al.*, 2008; Koshino *et al.*, 2008), response inhibition (Kana *et al.*, 2007), cognitive control (Solomon *et al.*, 2009), sentence comprehension (Just *et al.*, 2004; Kana *et al.*, 2006), planning (Just *et al.*, 2007), visuospatial processing (Damarla *et al.*, 2010; Liu *et al.*, 2011), during task-independant functional connectivity studies (Jones *et al.*, 2010) and in the resting state (Cherkassky *et al.*, 2006; Kennedy and Courchesne, 2008; Monk *et al.*, 2009; Weng *et al.*, 2010). More recently, evidence has emerged suggesting that it is not only fronto-posterior connections that are impaired in autism. Reduced functional connectivity has been also reported between the visual cortex and thalamus, striatum and cerebellum (Villalobos *et al.*, 2005), between the superior frontal gyrus and caudate (Turner *et al.*, 2006), between the fusiform gyrus and amygdala and posterior cingulate (Kleinhans *et al.*, 2008), within a motor network comprising the primary motor cortex, supplementary motor area, the cerebellum and the thalamus (Mostofsky *et al.*, 2009), between the prefrontal cortex and premotor and somatosensory cortices (Lombardo *et al.*, 2010), between the anterior cingulate and frontal eye fields (Agam *et al.*, 2010), and between the amygdala and temporal lobe (Monk *et al.*, 2010).

Independent Component Analysis (ICA) is a data-driven approach that identifies networks that show temporal coherence, by selecting brain regions whose activation time-series closely correlate. A recent study using ICA reported reduced functional connectivity between the precuneus and medial prefrontal cortex/anterior cingulate cortex and within a default mode network

that included the posterior cingulate, inferior parietal and medial prefrontal cortex (Assaf *et al.*, 2010). Structural Equation Modelling (SEM) is a statistical technique that has been used to investigate effective connectivity in the brain; the influential relationship between one brain region and another. Using SEM, Wicker et al (2008) reported weaker path coefficients (indicating reduced connectivity) in ASD in many prefrontal regions (Wicker *et al.*, 2008).

In addition to functional connectivity MRI research, PET, EEG and MEG studies have also reported reduced functional connectivity in autism. Reduced functional connectivity during a task of social cognition was shown using PET (Castelli *et al.*, 2002). EEG studies have demonstrated reduced long-range coherence in autism between frontal and posterior areas in the alpha band (Murias *et al.*, 2007), and the delta band (Coben *et al.*, 2008; Barttfeld *et al.*, 2011) and an MEG study also showed reduced coherence in ASD across a number of long and medium distance electrode pairs (Perez Velazquez *et al.*, 2009).

2.1.2. Increased functional connectivity in ASD

In parallel with this large body of literature suggesting a reduction in functional connectivity in ASD, some studies have also reported that *inter-regional* functional connectivity is increased in ASD relative to controls. Increased functional connectivity between the medial temporal lobe and various brain regions was reported during fearful face processing (Welchew *et al.*, 2005), among posterior regions (posterior cingulate and right temporal lobe/right parahippocampal gyrus) during the resting state (Monk *et al.*, 2009) and between the ventral prefrontal cortex and amygdala during an attention cueing task with emotional and neutral faces (Monk *et al.*, 2010). Increased functional connectivity has also been reported between the thalamus and several cortical areas (Mizuno *et al.*, 2006), between the caudate and frontal/cingulate/cuneus regions (Turner *et al.*, 2006) and between frontal and posterior regions (Noonan *et al.*, 2009).

A number of studies have also reported increased correlation of *intra-regional* functional activation, a finding that has been interpreted as evidence of local neural over-connectivity in ASD (e.g. (Belmonte and

Yurgelun-Todd, 2003; Belmonte *et al.*, 2004). It has been suggested that this hypothesized local overconnectivity could underpin some of the perceptual strengths observed in ASD (Mottron *et al.*, 2006). Functional MRI studies have reported increased local functional connectivity between fronto-fronto (Noonan *et al.*, 2009) and posterior-posterior pairs (Monk *et al.*, 2009; Noonan *et al.*, 2009). Regional homogeneity (ReHo) is an analytic method that gives an estimation of the local connectivity of every single voxel in the brain. Two recent studies revealed that there was both reduced *and* increased ReHo (local connectivity) in different brain regions in ASD. One study reported reduced ReHo in right superior temporal sulcus and cerebellum and frontal lobes, but increased ReHo in right thalamus, left inferior frontal gyrus, subcallosal gyrus and cerebellum (Paakki *et al.*, 2010) while another described reduced ReHo in frontal lobes and superior parietal lobe but increased ReHo in the temporal lobe (Shukla *et al.*, 2010b). These studies both provide evidence for local overconnectivity as they demonstrate increased local connectivity in the right thalamus, left inferior frontal gyrus, subcallosal gyrus, cerebellum (Paakki *et al.*, 2010) and temporal lobe (Shukla *et al.*, 2010b) in ASD. In addition, increased task-related activation in various brain regions has been reported in ASD relative to controls. Individuals with ASD showed increased activity in parietal regions during a selective attention task (Belmonte and Yurgelun-Todd, 2003), in primary visual cortex and extrastriate regions during a visual search task (Manjaly *et al.*, 2007), in the left inferior and orbital frontal gyrus during a motor inhibition task, in the parietal lobes during a set shifting task, in the left insula during an interference-inhibition task (Schmitz *et al.*, 2006), and in fronto-temporal regions when making mentalistic inferences from the eyes (Baron-Cohen *et al.*, 1999).

Other imaging methods have also provided evidence for increased intra-regional functional connectivity in ASD. Increased high-frequency gamma activity in ASD was reported during a resting state EEG study, a finding that implies excessive resting state connectivity (Orekhova *et al.*, 2007). Elevated short-range coherence in ASD has been reported during MEG in parietal areas (Perez Velazquez *et al.*, 2009) and during EEG studies in

both the theta band, most marked in short distance frontal and left temporal electrode pairs (Murias *et al.*, 2007) and the delta band (in lateral frontal electrode pairs) (Barttfeld *et al.*, 2011).

2.1.3. Summary of functional connectivity research in ASD

Autism is clearly associated with a marked disturbance of neural functional connectivity. There are a large number of studies demonstrating reduced functional connectivity between both long-range fronto-posterior connections and non fronto-posterior connections in ASD. There are also a number of studies that have revealed increased inter-regional and intra-regional functional connectivity in ASD. It would appear that functional connectivity abnormalities in ASD are widespread, affect multiple cognitive functions and can possibly be characterized by a signature of both increased and reduced patterns of connectivity.

2.2. Structural connectivity in ASD

Brain anatomical data, using a variety of measurement techniques including structural MRI, diffusion tensor imaging and post-mortem histological analysis, has revealed a number of abnormalities in autism that are suggestive of disrupted structural connectivity in autism. Findings of accelerated early brain growth, white matter enlargement, reduced structural organisation of white matter and abnormalities of neuronal subunits, called minicolumns, are all abnormalities that could compromise connections between brain regions in autism and lead to disrupted structural connectivity. While the majority of studies report white matter abnormalities that indicate reduced structural connectivity in autism, there is some evidence, in particular from post-mortem histological studies, to suggest the presence of structural over-connectivity in the disorder. The terms over- and under-connectivity indicate a relative difference in white matter integrity between groups.

2.2.1. Reduced structural connectivity in ASD – evidence from structural MRI and post-mortem brain studies

Evidence for reduced structural connectivity in ASD comes from structural MRI studies and head circumference studies. These methods have provided accumulating evidence that there are two phases of early brain

growth pathology in autism; a period of exceptionally rapid head growth during the first year of life followed by the slowing or arrest of growth during early childhood (Courchesne *et al.*, 2001; Courchesne *et al.*, 2003; Herbert, 2005; Lainhart *et al.*, 2006; Courchesne *et al.*, 2007). Research suggests that early brain overgrowth is not ubiquitous across the brain in autism. The frontal lobes, temporal lobes and amygdala have been identified as regions of peak overgrowth (Courchesne *et al.*, 2001; Carper *et al.*, 2002; Sparks *et al.*, 2002; Carper and Courchesne, 2005; Hazlett *et al.*, 2005). Increased early brain volume in autism appears to be driven mainly by disproportionate enlargement of white matter (Courchesne *et al.*, 2001; Herbert *et al.*, 2003), which has been localized to the radiate white matter, a white matter compartment corresponding to the corona radiata and subcortical white matter (Makris *et al.*, 1999), in all lobes (Herbert *et al.*, 2004; Herbert, 2005). The underlying neurobiology of this overgrowth is unknown. One theory holds that it may result from an excess of neurons in key frontal and temporal regions that mediate higher-order cognitive functions. The excess of neurons may be the result of a reduction in the normal process of synaptic pruning and apoptosis that occurs in the first two years of life or it may be due to excessive glial proliferation (Herbert, 2005). These excess neurons could lead to "exuberant local and short-distance cortical interactions impeding the function of large-scale, long distance interactions between brain regions" (Courchesne *et al.*, 2007) and interestingly, a recent post-mortem brain study found a 67% increase in the number of neurons in the prefrontal cortex of the brains of individuals with ASD relative to controls (Courchesne *et al.*, 2011). Although the abnormal brain growth trajectory in autism does not necessarily imply abnormal structural connectivity, there is some evidence to suggest that acceleration of brain growth may lead to different optimal connectivity patterns (Sporns and Zwi, 2004), see (Wass, 2011) for review. Larger brains tend to rely more on local than long-distance connections in part because of conduction delays and increased cellular costs (Lewis and Elman, 2008). Therefore in autism accelerated early brain growth may lead to greater reliance on local short-range neural connectivity and reduced reliance on global long-range connectivity. Results from a post-mortem brain study are in keeping with this hypothesis. Decreased myelin

thickness in axons in white matter of the orbitofrontal cortex may contribute to reduced long-range neural connectivity, and excessive number of thin axons linking neighbouring areas in white matter below the anterior cingulate cortex suggest that local short-range connectivity may be increased (Zikopoulos and Barbas, 2010).

Structural MRI studies have also identified abnormalities of the corpus callosum in autism that provide strong evidence for reduced structural connectivity in autism. Two meta-analyses of MRI studies examining corpus callosum area in autism have reported an overall reduction in corpus callosum size (Stanfield *et al.*, 2008; Frazier and Hardan, 2009) and this measure has been used as an index of general anatomical connectivity in the brain. Interestingly, four studies, from the same research group, have reported that measures of functional connectivity were positively correlated with corpus callosum size in autism (Cherkassky *et al.*, 2006; Kana *et al.*, 2006; Just *et al.*, 2007; Mason *et al.*, 2008) suggesting an intruiging link between brain structure and brain function.

2.2.2. Introduction to Diffusion Tensor Imaging (DTI)

The literature regarding the micro-structural properties of white matter in autism has expanded rapidly since the first DTI study in autism in 2004. Recent advances in analytic techniques for diffusion imaging data have facilitated investigation in this area. DTI is a non-invasive MRI method which can be used to study the neurobiological properties of white matter tracts in vivo (Catani *et al.*, 2008). DTI allows for the assessment of the underlying architectural organisation of white matter tracts through measurement of the restricted diffusion of water molecules in tissue (Basser and Jones, 2002; Jones and Leemans, 2011). It permits quantification of the directionality and coherence of neuronal fibres (Stieltjes *et al.*, 2001) and therefore represents an important method for measuring structural connectivity in humans (Stieltjes *et al.*, 2001; Alexander *et al.*, 2007b).

Physics of DTI

DTI is sensitive to the diffusion of water molecules in the body. When water molecule diffusion is not impeded by any barriers such as cell membranes, the diffusion occurs equally in all directions. In such a situation, if diffusion

from one particular point is measured, the water molecules will diffuse away from this central point in a sphere. This type of unhindered water diffusion is called "isotropic". In body tissue however, there are numerous barriers to the diffusion of water molecules (e.g. cell membranes or axonal myelin sheaths in brain white matter). When the diffusing water molecules meet such a barrier, the 'sphere' of diffusion will be disrupted, and diffusion becomes uneven. In this case, when measuring diffusion from a particular point, the mobility of the water molecules from the origin will now have a shape different to a sphere. This shape is often modelled as an ellipsoid, and the diffusion is termed "anisotropic". In white matter, the axonal myelin sheaths act as the principle barrier to the diffusion of water molecules. Bundles of axons allow parallel diffusion of water molecules along the orientation of the fibres, but restrict perpendicular diffusion. It is this phenomenon that permits DTI to distinguish white matter tracts in the brain. In major white matter tracts, where there are many bundles of myelinated axons clustered together, a high degree of anisotropic diffusion is expected.

Measures of white matter micro-structural organisation

DTI allows measurement of a number of diffusivity indices that relate to the the micro-structural organisation of brain white matter (Basser and Pierpaoli, 1996; Alexander *et al.*, 2007b). The most common indices are fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD).

FA is a ratio, where a value of "0" refers to a perfect sphere with pure isotropic diffusion, and a value of "1" refers to ideal linear diffusion. Typically, white matter tracts have an FA greater than 0.2. The measurement of the degree of anisotropy provides information about the structural integrity of the white matter; in conditions where the structure of the axon is disrupted, for example inflammation/trauma/tumour, the barrier to diffusion will be reduced, and anisotropy will be less than expected. Hence a reduction in FA is thought to be associated with disrupted organisation of white matter fibre tracts. Reduced FA may reflect demyelination, axonal damage or loss of white matter coherence (Basser,

1995; Basser and Pierpaoli, 1996; Werring *et al.*, 2000; Alexander *et al.*, 2007b).

While FA is very sensitive to micro-structural changes, it is not very specific about the type of change. It has been suggested that to better characterise the tissue microstructure, diffusion measures of axial diffusivity, radial diffusivity, and mean diffusivity should be measured in conjunction with FA (Alexander *et al.*, 2007b). The ellipsoid resulting from anisotropic diffusion of water molecules in tissue can be deconstructed into three principle eigenvalues (λ1, λ2 and λ3). The largest eigenvalue (λ1) is referred to as axial diffusivity and represents diffusion parallel to the axonal direction. Radial diffusivity (calculated by (λ2+λ3)/2) reflects diffusion perpendicular to the white matter tracts. Reduced axial diffusivity may indicate altered axonal integrity and increased radial diffusivity is generally accepted to be a sensitive marker for demyelination (Song *et al.*, 2005). These values are thought to allow detection of subtle structural abnormalities that cannot be detected by FA (Alexander *et al.*, 2007b). Mean diffusivity (calculated by (λ1+λ2+λ3)/3)) is a scalar value which refers to the total magnitude of diffusion in a voxel regardless of direction. An increase in mean diffusivity is thought to reflect oedema, demyelination and axonal loss (see (Alexander *et al.*, 2007b) for review).

The Westin measures of linear diffusion co-efficient (CL), planar diffusion co-efficient (CP) and spherical diffusion co-efficient (CS) are alternative measures of diffusion anisotropy (Westin *et al.*, 2002). These three measures describe the geometrical shape of the diffusion tensor, which is influenced by fibre density and diameter, degree of myelination and directional similarity of fibres in a voxel. The geometric nature of the measured diffusion tensor in a voxel provides a meaningful measure of tract organization (Westin *et al.*, 2002). Diffusion can be divided into three cases depending on the rank of the diffusion tensor. In the linear case, where λ1 >> λ2≈λ3, diffusion is mainly in the direction corresponding to the largest eigenvalue. CL is calculated from (λ1-λ2)/ λ1. It reflects the uniformity of tract direction within a voxel. This measure can be used for quantitative evaluation of fibre tract organization. When the measure of CL in a voxel is

greater than that of CP and CS, it implies that there is only one dominant fibre orientation (Vos *et al.*, 2012). In the planar case, where $\lambda1 \approx \lambda2 >> \lambda3$, diffusion is restricted to a plane that is spanned by the two eigenvectors corresponding to the two largest eigenvalues. CP is calculated from $(\lambda2-\lambda3)/\lambda1$. In voxels where CP is larger than CL and CS, it implies the presence of complex fibre architecture, i.e. the presence of more than one dominant fibre orientation within the voxel, including crossing, kinking, bending, splaying, twisting and bending configurations (Vos *et al.*, 2012). In the spherical case, $\lambda1 \approx \lambda2 \approx \lambda3$, diffusion is isotropic. A high CS may arise from the presence of multiple fibre populations, or from partial voluming with CSF (Vos *et al.*, 2012). Thus the interpretation of CS is more ambiguous that that of CL and CP. To date, the Westin measures have not been widely used in the diffusion MRI literature, however CL and CP provide valuable information about white matter micro-structural organisation. Therefore the use of these measures in conjunction with FA, MD, AD and RD allow for an improved characterization of white matter micro-structure.

2.2.3. Reduced structural connectivity in ASD – evidence from DTI

The majority of DTI studies investigating the structural organisation of white matter in autism have demonstrated white matter abnormalities that are likely to impact negatively on structural connectivity in the disorder. Three main approaches have been used for analysis of DTI data in these studies: voxel-based whole-brain and region of interest analyses, Tract Based Spatial Statistics analysis and DTI tractography analyses. The majority of the studies have typically reported reduced FA; some more recent studies have also described that reduced FA is accompanied by increased values of radial diffusivity and mean diffusivity and by reduced axial diffusivity. A review of the literature using voxel-based studies, TBSS and DTI tractography analyses in ASD research is presented below.

Voxel based studies

The majority of the earlier studies (2004 - 2010) that used DTI to investigate white matter pathology in ASD explored whole-brain white matter using voxel-based morphometry (VBM) style analyses. Such analyses are typically used to localize differences in grey matter density. Some voxel-

based studies also defined specific regions of interest (ROIs) in which they specifically probed for evidence of disrupted white matter in ASD (Alexander *et al.*, 2007a; Lee *et al.*, 2007a; Catani *et al.*, 2008; Sundaram *et al.*, 2008; Thakkar *et al.*, 2008; Pardini *et al.*, 2009).

In the first published DTI study in autism, Barnea-Goraly et al performed a whole-brain voxel-based analysis of DTI data and found reduced FA in a number of frontal and temporal brain regions that have been implicated in social cognition and theory of mind tasks including the fusiform gyrus, amygdala, superior temporal sulcus, temporoparietal junction, prefrontal cortex and anterior cingulate (Barnea-Goraly *et al.*, 2004). Disruption of white matter microstructure, characterised predominantly by reduced FA, in frontal and temporal brain regions in ASD has since been reported in a number of studies using a voxel-based approach (Lee *et al.*, 2007a; Sundaram *et al.*, 2008; Cheung *et al.*, 2009; Ke *et al.*, 2009; Pardini *et al.*, 2009; Bloemen *et al.*, 2010; Ingalhalikar *et al.*, 2010; Lange *et al.*, 2010; Noriuchi *et al.*, 2010). Abnormal white matter of the corpus callosum has also been found using whole-brain and ROI voxel-based approaches. A study examining the developmental trajectory of white matter in autism demonstrated reduced FA in areas within and near the corpus callosum in a group of children and adults (aged 10-35 years old). The authors of this study argued that reduced structural integrity of white matter persists into adulthood in individuals with autism (Keller *et al.*, 2007). Alexander et al (2007) selected the corpus callosum as a ROI for voxel-based analysis and found reduced FA, increased mean diffusivity and increased radial diffusivity in this region (Alexander *et al.*, 2007a). Similar results were reported by Shukla et al (2010) who found reduced FA and increased RD in the corpus callosum (Shukla *et al.*, 2010a). Reduced FA in the anterior cingulate was also reported in a voxel-based ROI analysis and interestingly, the reduced FA in this region was shown to correlate with higher levels of repetitive behaviour in adults with ASD (Thakkar *et al.*, 2008). This is one of very few studies attempting to relate brain structural abnormalities to brain function.

In summary, these voxel-based studies have identified disruption of white matter in numerous brain regions in autism. The majority of studies have

reported reduced organization of white matter in frontal and temporal lobes and in the corpus callosum.

Tract-based spatial statistics (TBSS)

More recently, a number of studies have used a newer approach, known as Tract-based Spatial Statistics (TBSS) (http://www.fmrib.ox.ac.uk/fsl/tbss/), that was specifically designed to resolve some of the methodological issues associated with whole-brain voxel-by-voxel analyses of diffusion weighted data. These will be discussed further in chapter 4. Essentially TBSS is an automated observer-independent method allowing voxel-wise comparisons of diffusion properties of white matter. Over the past two years a number of studies using TBSS have reported reduced organisation of white matter in a number of brain regions in autism. The most consistent white matter abnormality reported to date is of reduced FA, increased MD and increased RD in the corpus callosum [(Ameis *et al.*, 2011; Cheon *et al.*, 2011; Shukla *et al.*, 2011) but see (Weinstein *et al.*, 2011)], superior longitudinal fasciculus and inferior fronto-occipital fasciculus [(Ameis *et al.*, 2011; Jou *et al.*, 2011; Shukla *et al.*, 2011) but see (Bode *et al.*, 2011; Weinstein *et al.*, 2011)], internal capsule (Cheng *et al.*, 2010; Shukla *et al.*, 2011), anterior thalamic radiation (Cheon *et al.*, 2011; Shukla *et al.*, 2011) and uncinate fasciculus (Ameis *et al.*, 2011; Cheon *et al.*, 2011). Other findings from TBSS studies that are yet to be replicated include reduced FA, +/- increased MD and RD in the inferior longitudinal fasciculus, cingulum, corticospinal tract (Shukla *et al.*, 2011) corona radiata (Ameis *et al.*, 2011), and forceps minor (Jou *et al.*, 2011). These findings are discussed in detail in chapter 4.

DTI Tractography studies

Although voxel-based studies are informative about overall changes in white matter, they are unable to provide information about the micro-structural organisation of specific white matter tracts connecting brain regions. A technique that can partially overcome the limitations of traditional voxel-based approaches to DTI analysis is DTI-based tractography (Conturo *et al.*, 1999; Jones *et al.*, 1999; Basser *et al.*, 2000; Mori and van Zijl, 2002). DTI-based tractography is an analytic process that essentially uses the orientation of the diffusion profile to reconstruct the trajectories of white

matter fibre bundles. With recent advances in fibre-tracking software, investigators can now perform virtual in vivo dissections of white matter tracts, visualise white matter pathways in the brain and measure micro-structural organisation within specific tracts in the human brain (Catani *et al.*, 2008). The diffusion tractography approach has revolutionized our ability to investigate the role of specific white matter connections in the brain and how they relate to brain function, behaviour and disease.

Over the past four years, a number of DTI tractography studies have reported abnormalities of specific white matter tracts in individuals with ASD. There is evidence of disruption of white matter micro- and macro-structure in a number of major inter- and intra-hemispheric tracts including the corpus callosum, uncinate fasciculus, cingulum, arcuate fasciulus, inferior longitudinal fasciculus, fornix, inferior fronto-occipital fasciculus, anterior thalamic radiation, internal capsule and fronto-striatal tracts, which are discussed in detail below.

Corpus callosum (CC)

To date, eight diffusion tractography studies have reported abnormal white matter in the CC in autism. The results are quite mixed, however, and provide an unclear picture of the pathology. With regard to microstructure, two studies of children (aged 2.5 to 9 years) and adolescents (aged 14-17 years) have reported reduced FA (Kumar *et al.*, 2010) and reduced "Generalised FA" (a measure similar to FA) (Lo *et al.*, 2011) in the CC. In contrast, two studies of very young children aged <4 years (Weinstein *et al.*, 2011) and 6 months old (Wolff *et al.*, 2012) reported increased FA. Two other studies of older children (6-12 years) (Poustka *et al.*, 2011) and adults (Thomas *et al.*, 2011) failed to find any difference in FA in the CC. Other white matter abnormalities identified in the CC in ASD imply compromise of macro-structural organisation. Reduced volume of the CC has been reported (Hong *et al.*, 2011) and interestingly, a reduction in the volume of the forceps minor and body of CC in adults with ASD showed negative correlation with the presence of repetitive and stereotypical behaviour (Thomas *et al.*, 2011). Another study however, reported increased length and fibre density of the CC in children with autism (Kumar *et al.*, 2010).

Together, these studies provide an indication that CC micro- and macro-structure is disrupted in autism and that the perturbation in white matter may be age-related.

Uncinate fasciculus (UF)

Results from diffusion tractography studies that have examined the uncinate fasciulus in autism are quite mixed and, as for the corpus callosum, no clear picture of the underlying pathology has emerged. With regard to micro-structural organisation of the UF, reduced FA has been reported in two studies (Kumar *et al.*, 2010; Poustka *et al.*, 2011) and reduced FA in the left UF showed negative correlation with ADI-R measures of communication and interaction (Poustka *et al.*, 2011). Reduced left lateralization of FA in the UF in autism has also been described (Lo *et al.*, 2011). In contrast to these findings of reduced FA, increased FA in the UF was found in 6 month old infants who developed autism, but no difference in the FA of this tract was present at 12 or 24 months (Wolff *et al.*, 2012) or in adults with ASD (Thomas *et al.*, 2011). Abnormalities of MD have also been identified in the UF (Pugliese *et al.*, 2009). From a macro-structural perspective, a reduced number of streamlines, which is a possible surrogate of tract volume (Pugliese *et al.*, 2009), increased left lateralization of volume, as measured by the number of streamlines and number of voxels (Thomas *et al.*, 2011), and increased curvature at the fronto-temporal junction (Jeong *et al.*, 2011) has been reported in the UF in autism.

Cingulum

A study of very young children with autism (mean age 3.2 years) found increased FA in the cingulum (Weinstein *et al.*, 2011), whereas a study of slightly older children (range 2.5-9 years) found reduced FA in this tract (Kumar *et al.*, 2010). Another study found no between-group difference in a measure similar to FA (Generalised Fractional Anisotropy), but adolescents with ASD in this study showed an absence of left lateralization of GFA in the cingulum (Lo *et al.*, 2011). Structurally, findings of increased number of streamlines in right and left cingulum in adults with ASD (Pugliese *et al.*,

2009) and increased fibre density in left cingulum in young children with ASD (Kumar *et al.*, 2010) have been reported.

Other tracts

A small number of tractography studies have isolated other major white matter tracts in ASD. Reduced left lateralization (Fletcher *et al.*, 2010; Lo *et al.*, 2011), increased curvature (Jeong *et al.*, 2011) and reduced FA (Kumar *et al.*, 2010) of the arcuate fasciculus have been reported in individuals with autism. Relative to controls, increased left lateralization of volume was reported in the inferior fronto-occipital fasciculus in adults with ASD (Thomas *et al.*, 2011), however two other studies failed to find any abnormalities of this tract (Pugliese *et al.*, 2009; Kumar *et al.*, 2010). Increased FA in the inferior longitudinal fasciculus was reported in 6 month old infants with autism (but not 12 or 24 month olds) (Wolff *et al.*, 2012). Two other studies failed to find differences of FA in this tract, but described increased left lateralization of tract volume (Thomas *et al.*, 2011) and increased number of streamlines in ASD (Pugliese *et al.*, 2009). An increase in FA was reported in the left fornix in infants (6 months) who developed ASD but there was no between-group difference in FA at 12 and 24 months (Wolff *et al.*, 2012), nor was there a difference in FA in this tract in older children (6-12 years) with ASD (Poustka *et al.*, 2011). Infants with ASD showed increased FA at 6 months but reduced FA at 24 months in the anterior and posterior limb of the internal capsule and reduced FA at 12 and 24 months in the left anterior thalamic radiation (Wolff *et al.*, 2012). Other findings from diffusion tractography studies include reduced FA in individuals with ASD in short intracerebellar fibres (Catani *et al.*, 2008), in hippocampo-fusiform and amygdalo-fusiform pathways (which was correlated with performance IQ and facial recognition scores) (Conturo *et al.*, 2008), in fibre tracts between the inferior frontal lobe and fusiform gyrus / inferior frontal lobe and medial temporal gyrus (Sahyoun *et al.*, 2010) and in white matter tracts connecting the putamen to the frontal cortex (Langen *et al.*, 2012).

2.2.4. Increased structural connectivity in ASD – evidence from structural MRI

A number of studies using functional MRI, EEG, MEG and regional homogeneity analyses have revealed increased inter-regional and intra-regional functional connectivity in ASD. Structural MRI, DTI and post-mortem histological studies have also provided some, albeit rather limited, evidence to suggest that there may be increased structural connectivity in autism. Support for increased connectivity in autism comes from structural MRI studies that have reported increased volumes in radiate white matter (Herbert *et al.*, 2003; Herbert *et al.*, 2004; Herbert, 2005; Ke *et al.*, 2009). The increase in radiate white matter may result in a bias toward local, short-range neural connectivity (Courchesne *et al.*, 2007) and increased intra-regional structural connectivity.

2.2.5. Increased structural connectivity in ASD – evidence from DTI

While the majority of DTI studies in autism have reported reductions in FA in white matter, some have also found increases in FA. A large prospective study recently examined white matter fibre tract organization from 6-24 months in high-risk infants who developed ASDs by 24 months (Wolff *et al.*, 2012). Results showed that there was abnormal development of white matter in the children who developed autism. Relative to controls, FA was increased at 6 months, but reduced at 24 months in a number of major white matter tracts. These results suggest that abnormal development of white matter pathways may precede the emergence of autistic symptoms. Two other DTI studies in young children (<4 years) with autism (Ben Bashat *et al.*, 2007; Weinstein *et al.*, 2011) also reported increased FA in the radiate white matter (Ben Bashat *et al.*, 2007), the corpus callosum, superior longitudinal fasciculus and cingulum (Weinstein *et al.*, 2011). Given the recent findings of (Wolff *et al.*, 2012) it is possible that the finding of increased FA in these two studies is related to the young age of the study participants. A number of studies in older children (>4 years) however have reported increased FA in combination with reduced FA elsewhere in the brain. A study that used TBSS to analyse DTI data from older children (age range 10-18 years), also reported increased FA (and reduced RD) in the frontal lobe, right cingulate gyrus, bilateral insula, right superior temporal

gyrus and bilateral middle cerebellar peduncle (Cheng *et al.*, 2010). The authors report an age-related loss of FA in the ASD group indicating abnormal neurodevelopment of the frontal lobe in adolescence in ASD. This suggests that individuals with ASD might fail to undergo complete maturation of white matter tracts, especially in the frontal lobe. A subsequent, similar TBSS study of older children (mean age 14.7 autism/14.5 controls) found increased FA in autistic individuals in the optic radiation and right inferior fronto-occipital fasciculus (Bode *et al.*, 2011) and whole-brain voxel-based DTI studies of older children have reported increased FA in autism in the right inferior frontal gyrus and left occipital lobe (Cheung *et al.*, 2009), in the right middle temporal gyrus and right frontal lobe (Ke *et al.*, 2009) and in temporal white matter and in the uncinate fasciculus (Sahyoun *et al.*, 2010).

The significance of the increased FA reported in these studies remains to be determined. Greater FA may reflect increased myelination of white matter fibres, increased numbers of myelinated fibres, smaller axonal diameter or reduced neural branches within the MRI voxel (Beaulieu, 2002; Hoeft *et al.*, 2007). Thus increased FA may indicate increased structural connectivity. Increased FA could also be artefactual, resulting from excessive partial volume averageing from multiple, differently oriented, fibres within a voxel (Le Bihan, 1995; Beaulieu, 2002; Schwartz and Hackney, 2003). The difficulties with interpretation of FA and other diffusion measures will be discussed in detail in chapter 4.

2.2.6. Increased structural connectivity in ASD – evidence from post-mortem brain studies

Hutsler and Zhang (Hutsler and Zhang, 2010) examined the expression of dendritic spines on pyramidal cells in the superficial and deep cortical layers of frontal, temporal and parietal lobes. This method has been used to assess the quantity and distribution of connections onto individual neurons and spine density has been used as an index of excitatory connectivity (Fiala *et al.*, 2002). Relative to controls, individuals with autism had increased synaptic spine density on cortical pyramidal cells, predominantly within layer II, a relatively late-maturing cortical layer that establishes

synaptic connections postnatally. Interestingly, pyramidal cells in layer II are involved in interconnectivity between cortical regions within a hemisphere (Hutsler and Zhang, 2010). The authors suggest that their finding indicates important alterations to cortical circuitry in autism and that it is in keeping with a theory of altered cortical connectivity in autism. They do point out, however, that they did not study whether inhibitory synapse distribution was also modified on the cell surface in these cases. Thus while this study offers anatomical evidence for increased structural connectivity in autism, it does not provide a full picture of histological abnormalities.

Casanova et al (2002, 2006) examined minicolumns in the brains of individuals with autism (Casanova *et al.*, 2002) . Minicolumns are basic functional units of the brain that organize neurons in cortical space. They found smaller, more numerous, more densely packed minicolumns in individuals with autism and suggested that their findings could reflect a bias towards shorter connecting fibres in autism, which would favour local computation at the expense of inter-regional and callosal connectivity (Casanova *et al.*, 2006). The finding of increased numbers of thin short-range axons in the orbitofrontal cortex of individuals with ASD is in keeping with this theory and suggests that intra-regional connectivity may be increased in the disorder (Zikopoulos and Barbas, 2010).

2.2.7. Summary of structural connectivity research in ASD

Results from voxel-based, TBSS and tractography analyses of DTI data indicate widespread reduction in micro- and macro-structural organization of white matter, a finding that suggests that structural connectivity might be compromised in ASDs. From an anatomical perspective, the most compelling evidence for increased connectivity in ASD comes from post-mortem histological studies. Results from structural MRI studies and DTI studies are more mixed and do not show consistent evidence for increased structural connectivity in autism.

3. Visuospatial processing in autism

To investigate the theory of abnormal cortical connectivity from a functional perspective, this study examines functional connectivity in ASD during a

visuospatial processing task. An introduction to visuospatial processing, including findings from behavioural and neuroimaging research in ASD is provided in this section.

Visuospatial processing is a term that refers to the cognitive processing of visual and spatial information. Although the majority of studies in autism tend to focus on the deficits associated with the disorder, individuals with autism often present with isolated skills in certain cognitive domains (Mottron *et al.*, 2006), a feature of the condition that was described as far back as 1943 as "islets of ability" (Kanner, 1943). On a variety of behavioural tests of visuospatial cognitive function, people with autism have been reported to demonstrate superiority over typically developing controls [e.g. (Shah and Frith, 1983; Jolliffe and Baron-Cohen, 1997; Plaisted *et al.*, 1998a; O'Riordan *et al.*, 2001; Caron *et al.*, 2004; Caron *et al.*, 2006; Dawson *et al.*, 2007; Joseph *et al.*, 2009; Soulieres *et al.*, 2011)]. A number of more recent neuroimaging studies, however, have reported intact visuospatial processing from a behavioural perspective but have also shown differential brain activity and connectivity between ASD and control groups [e.g. (Ring *et al.*, 1999; Silk *et al.*, 2006; Lee *et al.*, 2007b; Manjaly *et al.*, 2007; Damarla *et al.*, 2010)]. These findings suggest that visuospatial processing in autism may be accomplished in an atypical way.

3.1. A review of behavioural and neuroimaging studies of visuospatial processing in autism

The following section provides a detailed review of the behavioural and neuroimaging studies of visuospatial processing in autism. A variety of neuropsychological paradigms have been used to test visuospatial function including visual search tasks, the Embedded Figures Test, the Block Design subtest of the Wechsler Intelligence Scale, pattern discrimination tasks and mental imagery tasks.

3.1.1. Visual search tasks

Individuals with ASD have repeatedly shown enhanced performance on simple visual search tasks (Plaisted *et al.*, 1998a; O'Riordan and Plaisted, 2001; O'Riordan *et al.*, 2001; O'Riordan, 2004; Kemner *et al.*, 2008; Joseph *et al.*, 2009). These tasks typically involve an observer looking for a target

item among an array of distractor items and responding by indicating whether a target is present or absent. It has been suggested that visual search superiority in ASD may stem from enhanced visual perceptual abilities, e.g. (Kemner *et al.*, 2008; Joseph *et al.*, 2009).

3.1.2. Embedded Figures Test

A similar advantage has been reported for the more complex Embedded Figures Test (EFT), a test requiring the observer to locate a simple target shape hidden within a larger, complex, meaningful figure (Witkin *et al.*, 1971). Almost 30 years ago, Shah and Frith first reported that when tested on the EFT, children with autism were significantly more competent than control children (Shah and Frith, 1983). Superior performance on the EFT has since been replicated in a number of studies (Jolliffe and Baron-Cohen, 1997; Jarrold *et al.*, 2005; de Jonge *et al.*, 2006). One account for EFT superiority in autism is that there is enhanced local perceptual processing in early visual areas. A number of fMRI studies have investigated neural correlates of the EFT in autism and findings are in keeping with this theory. Individuals with ASD appear to rely heavily on visual systems for object feature analysis whereas controls involve working memory systems to a greater degree (Ring *et al.*, 1999; Lee *et al.*, 2007b; Manjaly *et al.*, 2007; Damarla *et al.*, 2010). These findings suggest that the two groups may employ qualitatively different cognitive strategies during EFT performance. Long-range fronto-posterior functional connectivity was also reduced in ASD during the EFT (Damarla *et al.*, 2010). While aberrant fronto-posterior connectivity has been linked to many of the core deficits in autism, it is possible that it confers relative advantage during lower-level perceptual tasks such as EFT performance, where integration of top-down, executive processes with visuospatial regions might not be beneficial. Individuals with ASD may benefit from reduced "interference" from top-down / global processes during tasks primarily requiring low-level visual processing (Liu *et al.*, 2011).

3.1.3. Block design subtest of the Wechsler Intelligence test

Another well-replicated finding of enhanced visuospatial performance in autism is that of superior performance on the Block Design subtest of the

Wechsler Intelligence Scale (Shah and Frith, 1983; Asarnow *et al.*, 1987; Shah and Frith, 1993; Happe, 1994; Siegel *et al.*, 1996; Caron *et al.*, 2006). Caron et al (2006) suggested that this observed Block Design peak in autism, where performance on the Block design subtest of the Wechsler Intelligence Scale exceeded that expected from overall Wechsler IQ, resulted from a combination of locally oriented processing and superior perceptual functioning (Caron *et al.*, 2006). The authors hypothesised that superior visual perceptual functioning might have resulted from 'overfunctioning' of the early visual cortex (V1), leading to superior visual input to the subsequent flow of visual information. The authors mentioned a number of the possible neurobiological factors that might contribute to 'overfunctioning' of V1 and atypical neural connectivity. These included long-range underconnectivity and short-range overconnectivity, both of which are held to play a crucial pathophysiological role in autism.

3.1.4. Pattern discrimination

Enhanced pattern discrimination in autism has also been described in a number of studies. Plaisted et al (1998) reported that adults with autism showed superior pattern discrimination in a perceptual learning task (Plaisted *et al.*, 1998b). In a study assessing spatial abilities, individuals with autism showed superior performance on tasks involving maps (Caron *et al.*, 2004). The authors suggested that there was "enhanced discrimination, detection and memory for visually simple patterns, which may account for the superior performance of persons with autism on visuo-spatial tasks that involve pattern recognition" (page 467). They suggested that this enhanced pattern discrimination might be associated with increased functioning of the occipito-temporal pathway but neural correlates were not investigated in this study.

Raven's progressive matrices are non-verbal multiple-choice tests of reasoning in which an individual is asked to identify the missing element that completes a pattern. Although this task is considered primarily a test of "fluid intelligence", there is a significant visuo-spatial processing component to the task in terms of pattern identification. Dawson et al described that children with autism scored significantly more highly on Raven's progressive

matrices than predicted by their Wechsler IQ (Dawson *et al.*, 2007). Similar findings were reported in a subsequent study in which individuals with Asperger syndrome showed superior performance on this task relative to age and IQ matched controls (Hayashi *et al.*, 2008). An fMRI study using Raven's progressive matrices demonstrated that individuals with autism displayed relatively increased task-related activation in extrastriate areas and decreased activity in the lateral prefrontal cortex and medial posterior parietal cortex (Soulieres *et al.*, 2009). The authors suggested that visual processing might play a more prominent role in reasoning in individuals with autism than controls (Soulieres *et al.*, 2009).

3.1.5. Mental imagery

A recent study investigating mental imagery in autism found that individuals with autism were more accurate in formation and comparison of mental images than a non-autistic group. A subgroup of individuals with autism demonstrated a "Block Design Peak", in which they demonstrated a higher than expected score on the Block Design subtest of the Wechsler Intelligence Scales, relative to their overall IQ estimates. This Block Design Peak subgroup was both faster and more accurate on a mental rotation task than those with autism but without the Block Design Peak (Soulieres *et al.*, 2011). The authors proposed that an advantage in perceptual processing and strengths in veridical mapping might explain these processing advantages in mental imagery. Veridical mapping is the detection of similarity among visual patterns. In terms of the neural correlates of mental imagery in autism, two fMRI studies have reported greater task-related activity in posterior brain regions associated with visual mental imagery and reduced activity in prefrontal areas associated with working memory (Kana *et al.*, 2006; Silk *et al.*, 2006). Kana et al (2006) also described reduced fronto-posterior functional connectivity during a mental imagery/sentence comprehension paradigm. These neuroimaging results are similar to those from fMRI studies investigating neural correlates of the EFT in autism – where individuals with autism show greater activation of visual regions whereas controls involve frontal executive systems to a greater degree.

3.1.6. Mental rotation

Mental rotation is a visuospatial cognitive function that involves the process of imagining an object rotated into a different orientation in space (Shepard and Metzler, 1971). In a classic mental rotation task, the participant is asked to compare images of two 3-dimensional objects and decide whether they are the same or mirror images of each other. The objects are rotated in relation to each other and the degree of rotation can vary from 0 to 180 degrees. The participant's response time and accuracy in distinguishing mirrored from non mirrored pairs is recorded. There are a number of cognitive functions involved during a typical mental rotation task. First, a mental image of the object must be created and this involves visual perceptual processing and stimulus encoding. Second, the participant must rotate the object mentally until a comparison can be made with the second object. Third, once the comparison is made the participant decides whether the objects are the same or not, and they then respond with their decision (Johnson, 1990).

There are a very limited number of studies investigating mental rotation in autism. One behavioural study reported that people with ASD excel specifically at non-rotational aspects (i.e. perceptual processing, stimulus encoding, comparison, decision making, response preparation) of mental rotation (Falter *et al.*, 2008). Another behavioural study found that children with autism showed superior mental rotation performance compared with typically developing children when matched on verbal mental age (Hamilton *et al.*, 2009). More recently, it was reported that individuals with autism who showed a Block Design peak showed faster response times and increased accuracy on a mental rotation task (Soulieres *et al.*, 2011).

A number of neuroimaging studies have investigated the neural correlates of mental rotation in neurotypical populations and the neural network required for successful mental rotation is well established. In a recent meta-analysis of neuroimaging studies on mental rotation, Zacks et al (2007) found that the superior parietal lobe, frontal lobe and inferotemporal cortex were the most consistently activated brain regions during mental rotation. Activity in the parietal cortex appeared to be more consistent in the right hemisphere

but activity in the frontal cortex appeared to be more consistent in the left hemisphere (Zacks, 2007). Many of the studies included in the meta-analysis reported significant foci of activation in the superior parietal cortex, a brain area known to implement spatial maps, and the intraparietal sulcus, extending into the transverse occipital sulcus including BA 7, 19, 39 and 40. It has been suggested that the parietal lobes are responsible for the rotational aspects of mental rotation (Gill *et al.*, 1998; Hugdahl *et al.*, 2006). Bilateral activation of the precentral sulcus and regions in the precentral cortex were also widely reported in the studies in the meta-analysis. These brain regions have been associated with motor planning and execution, and brain activity in these regions during mental rotation may reflect motor simulation (Zacks, 2007). The meta-analysis also identified significant regions of activation in the prefrontal cortex during mental rotation. The specific foci of activation that were identified (BA 44 and 45) have been implicated both in speech control and also in motor control and imitation (Rizzolatti *et al.*, 1996) and Zacks et al (2007) suggest that the activation in this region may reflect the use of motor simulation in some mental rotation tasks. The frontal lobes may mediate the non-rotational aspects of mental rotation including encoding and decision-making processes (Gill *et al.*, 1998). Using Structural Equation Modelling (SEM), it has been proposed that the pathway for the neural network subserving mental rotation involves parallel processing with feedback connections between the nodes of the network, including the primary visual area, extrastriate cortex, parietal regions, and premotor cortex (Ecker *et al.*, 2008).

To date, only one small neuroimaging study has investigated mental rotation in ASD (Silk *et al.*, 2006). This study found that individuals with autism (n=7) showed significant differences in brain activation compared with controls (n=9). Specifically the authors reported that the ASD group showed a reduction in activation in prefrontal regions including the lateral and medial premotor cortex, dorsolateral prefrontal cortex, anterior cingulate gyrus and caudate nucleus. The authors suggest that frontostriatal networks may be dysfunctional in ASD. Given the small sample size and consequent limited

power, further exploration of this question in an appropriately powered sample is required.

3.2. Summary of visuospatial processing in autism

Atypical visuospatial processing in autism is characterised either by enhanced performance on visuospatial tasks or preserved (normal) visuospatial function coupled with abnormal neural activation. The underlying pathophysiology, however, remains poorly understood.

4. Outstanding issues, aims and hypotheses of this thesis

4.1. Outstanding issues

There is strong evidence from both functional and structural neuroimaging studies to suggest that neural connectivity in ASD is abnormal. There is a striking lack of research attempting to investigate firstly the association between functional connectivity and structural connectivity, and secondly how abnormal connectivity influences behaviour. Investigation of structural connectivity, functional connectivity and behaviour in the same study population provides a unique opportunity to interrogate the relationships between brain structure, function and information processing in ASD.

Atypical visuospatial processing is commonly described in autism spectrum disorders (ASDs), however the specific neurobiological underpinnings of this phenomenon are poorly understood. A variety of neuropsychological paradigms have been used to test visuospatial processing in ASD but very little is known about the visuospatial process of mental rotation in the disorder. In typically developing populations, the neural correlates of mental rotation are well established and it is known that successful mental rotation relies on the integration of information within a distributed neural network. Consequently, mental rotation paradigms provide an ideal neuroimaging opportunity to assess the functioning and connectivity of a distributed brain network. Surprisingly, given the extensive evidence for aberrant connectivity in autism, there are no studies investigating functional connectivity during mental rotation in individuals with ASD.

With regard to structural connectivity, at the whole-brain level, numerous studies have identified abnormalities of white matter micro-structure in ASD but there is no clear picture of the underlying neural pathology. This may in part be due to differing protocols for acquisition and processing of diffusion MRI data. Advanced processing methods that will improve the reliability of whole-brain white matter analysis are now available but have not previously been used in ASD research. The most recent studies examining structural connectivity in ASD have focused on isolation of specific white matter tracts in the brain using diffusion tensor tractography (DTT) approaches. Although a tractography approach offers a number of advantages over whole-brain analysis, new research has suggested that limitations of the DTT approach are likely to have profound implications for the interpretation of results from any study using this methodology. An advanced tractography technique using Constrained Spherical Deconvolution (CSD) has been developed to overcome limitations of the DTT approach but CSD-based tractography has not yet been used in ASD research.

Despite the extensive evidence for disrupted functional and structural connectivity in ASD, no previous studies have attempted to integrate these types of connectivity analysis. Multimodal (structure/function) connectivity studies in neurotypical populations have revealed strong relationships between white matter organization, functional connectivity and behaviour and it is hoped that this approach will provide novel insights into the underlying pathophysiology of ASD.

4.2 Aims

1. To characterise the neuropsychological function of mental rotation in a well-defined sample of individuals with ASD and to investigate differences in brain activity between individuals with ASD and controls during a mental rotation task.
2. To determine whether there is abnormal functional connectivity within the neural network required for mental rotation in individuals with ASD compared with controls.

3. To analyse a high quality DTI dataset using advanced processing techniques to investigate whether, at the whole-brain level, there are true differences in white matter organisation between individuals with ASD and controls.
4. To comprehensively assess white matter organisation in specific tracts that are important in visuospatial processing using an advanced tractography technique (Constrained Spherical Deconvolution based tractography) that has not previously been used in ASD research.
5. To relate functional connectivity to structural connectivity, using a novel analytical approach, to investigate whether abnormal functional connectivity is associated with abnormal structural connectivity and to examine relationships between brain white matter structure, functional connectivity and behaviour.

4.3. Hypotheses

In relation to aims 1 and 2, the hypothesis was that mental rotation abilities would be preserved or enhanced in ASD but that brain activation and functional connectivity during mental rotation would be atypical in ASD. In relation to aim 3, the hypothesis was that whole-brain analysis of diffusion MRI data would reveal reduced organisation of white matter micro-structure in regions of the frontal and temporal lobes and in the corpus callosum in individuals with ASD. In relation to aim 4, the hypothesis was that major white matter tracts involved in visuospatial processing would show disrupted white matter micro-structure, and that these abnormalities would be related to visuospatial processing speed. In relation to aim 5, the hypothesis was that there would be an association between functional and structural connectivity in ASD; specifically that there would be abnormal white matter micro-structure in tracts that directly connected pairs of brain regions with abnormal functional connectivity and that there would be significant correlations between micro-structural organization of white matter, functional connectivity and behaviour.

2. Mental rotation in ASD

1. Introduction

1.1. Functional MRI

Functional Magnetic Resonance Imaging (fMRI) is a type of specialized Magnetic Resonance Imaging method that measures change in blood oxygenation over time. This is known as the haemodynamic response and it is closely related to neural activity in the brain (Roy and Sherrington, 1890). fMRI allows the detection of areas of the brain that are active at a particular time point, and thus can be used to map the neural regions that are involved in specific cognitive functions.

1.1.1. Physics of functional MRI

When neurons are active, their oxygen consumption increases (Raichle and Mintun, 2006). The increased oxygen requirement leads to a corresponding increase in blood flow to the active neural regions. Blood releases oxygen more rapidly to active than inactive neurons, resulting in local changes in the ratio of oxyhaemoglobin to deoxyhaemoglobin. When the haemoglobin molecule is oxygenated, it is diamagnetic, but when deoxygenated it is paramagnetic (Pauling and Coryell, 1936). Diamagnetic materials are repelled by magnetic fields, and paramagnetic materials are attracted to magnetic fields. This difference in magnetic susceptibility causes oxygenated and deoxygenated blood to have different magnetic resonance (MR) signals. This difference in MR signal between oxygenated and deoxygenated blood is called the BOLD contrast (Blood Oxygen Level Dependant contrast) and it forms the basis of functional magnetic resonance imaging, allowing the detection of active versus inactive neural regions (Ogawa *et al.*, 1990a; Ogawa *et al.*, 1990b). Higher BOLD signal results from increases in the concentration of oxyhaemoglobin and increases in cerebral blood flow; conversely lower BOLD signal results from decreased cerebral blood flow and increased deoxyhaemoglobin concentration.

During an fMRI scan, changes in BOLD contrast are measured over time. Typically the person is given a task to compete in the scanner, which is

designed to test a discrete cognitive function or action. The BOLD signal changes are very small, but with multiple repetitions of the thought process or action during the scan, analytic techniques allow identification of brain regions that were significantly more active. A more active brain region has more cerebral blood flow and more oxygenated haemoglobin during a particular cognitive function or action compared with when a person is at rest or is performing some other cognitive function or action.

1.1.2. Functional MRI studies in ASD

Numerous functional MRI studies have investigated brain activity in ASD during a wide variety of cognitive tasks. ASDs are characterized by deficits in the three core domains of social interaction, verbal and non-verbal communication and repetitive or stereotyped behaviours. Functional MRI research has tended to study cognitive functions in these core domains, seeking to increase our understanding of the neuropsychological deficits in ASD. Abnormalities in brain activity during a variety of different cognitive functions in these domains have been described [e.g. see (Verhoeven *et al.*, 2009; Philip *et al.*, 2012) for a review]. Although the majority of studies in ASD tend to focus on deficits associated with the disorder, an increased understanding of the neural correlates of a cognitive domain in which autistic individuals demonstrate isolated skills or preserved function may provide important insights into the overall pathophysiology of the disorder, and may possibly lead to the development of future therapeutic approaches.

1.2. Visuospatial processing in ASD

This study investigates visuospatial processing in ASD using fMRI. Atypical visuospatial processing in ASD is characterised either by enhanced performance on visuospatial tasks or preserved (normal) visuospatial function coupled with abnormal neural activation. The underlying pathophysiology, however, remains poorly understood.

As detailed in chapter 1 section 3.1., enhanced visuospatial processing in ASD has been described in behavioural studies during a variety of cognitive tasks including the Embedded Figures Test (Shah and Frith, 1983; Jolliffe and Baron-Cohen, 1997; Jarrold *et al.*, 2005; Pellicano *et al.*, 2005; de

Jonge *et al.*, 2006), visual search tasks (Plaisted et al., 1998; O'Riordan and Plaisted, 2001; O'Riordan et al., 2001; O'Riordan, 2004; Kemner et al., 2008; Joseph et al., 2009), the Block Design subtest of the Wechsler Intelligence tests (Shah and Frith, 1983; Asarnow *et al.*, 1987; Shah and Frith, 1993; Happe, 1994; Siegel *et al.*, 1996; Caron *et al.*, 2006), pattern discrimination (Plaisted et al., 1998a; Caron et al., 2004), Raven's progressive matrices (Dawson *et al.*, 2007; Hayashi *et al.*, 2008) and mental imagery tasks (Soulieres *et al.*, 2011). In contrast with this finding of superior behavioural performance, a number of recent neuroimaging studies have reported that behaviourally, visuospatial processing in ASD is comparable to controls. Despite this apparent comparable level of behavioural performance, brain activity and connectivity differ markedly between ASD and control groups (Ring *et al.*, 1999; Silk *et al.*, 2006; Lee *et al.*, 2007; Manjaly *et al.*, 2007; Damarla *et al.*, 2010) indicating atypical visuospatial function in autism at a neural level.

Cognitive theories addressing atypical visuospatial processing in ASD include the Weak Central Coherence (WCC) theory and the Enhanced Perceptual Functioning theory. The WCC theory proposes that there is superior local processing in autism, possibly combined with a deficit in global integration of information (Happe and Frith, 2006). The Enhanced Perceptual Functioning theory holds that there are enhanced perceptual operations and superior locally oriented processing in ASD (Mottron *et al.*, 2006). No specific neurobiological explanation is offered for either theory, however, and there are a very limited number of neuroimaging studies investigating the neural correlates of visuospatial processing in autism. Taken together, this small number of fMRI studies have suggested that individuals with autism may employ qualitatively different cognitive strategies to typically developing controls for visuospatial processing. During performance of the EFT (Ring *et al.*, 1999; Lee *et al.*, 2007; Manjaly *et al.*, 2007; Damarla *et al.*, 2010), Raven's progressive matrices (Soulieres *et al.*, 2009) and mental imagery tasks (Kana *et al.*, 2006; Silk *et al.*, 2006), individuals with autism have shown intact behavioural performance relative to controls but greater task-related activation of posterior regions and

reduced activation of frontal brain areas. Thus, individuals with autism appear to rely heavily on visual systems for object feature analysis while controls involve frontal executive systems to a greater degree.

1.3. Mental rotation in ASD

Mental rotation, the process of imagining an object rotated into a different orientation in space (Shepard and Metzler, 1971) is a visuospatial cognitive function that has received surprisingly little attention in autism research. There is an interesting relationship between testosterone levels, mental rotation and autism. Higher testosterone levels have been implicated in the aetiology of autism (Baron-Cohen *et al.*, 2005), and have also been shown to impact favourably on mental rotation (Grimshaw *et al.*, 1995; Hooven *et al.*, 2004; Sanders *et al.*, 2005; Peters *et al.*, 2007). A handful of behavioural studies have suggested that mental rotation may be enhanced in ASD (Falter *et al.*, 2008; Hamilton *et al.*, 2009; Soulieres *et al.*, 2011) but the neural correlates of this cognitive function have not been well established in autism. To date, there has been just one small-sample neuroimaging study (Silk *et al.*, 2006) that reported intact behavioural performance but a reduction in prefrontal and striatal activation in the ASD group during mental rotation.

1.4. Aims and hypothesis

The aims of this study were to (a) characterise the neuropsychological function of mental rotation in a well-defined sample of individuals with ASD and (b) to investigate brain activity during mental rotation in order to increase understanding of the pathophysiology of atypical visuospatial processing in ASD. We hypothesised that mental rotation abilities would be preserved or enhanced in ASD based on the few previous studies investigating mental rotation in ASD (Silk *et al.*, 2006; Falter *et al.*, 2008; Hamilton *et al.*, 2009; Soulieres *et al.*, 2011). In addition, based on the findings of previous neuroimaging studies of visuospatial processing, we hypothesised that brain activation during mental rotation would be atypical in ASD.

2. Methods

2.1. Participants

Twenty-two right-handed male individuals with ASD (mean age 17.56, mean IQ 105.95) and twenty-two right-handed age- and IQ-matched male neurotypical controls (mean age 17.51, mean IQ 110.50) were included in the analysis (for both age and IQ there were no significant group differences, $p > 0.05$) (see Table 1). Data from three other participants with ASD (and their matched control participants) were discarded due to excessive head motion or other technical problems.

Participants with ASD were recruited from an existing autism genetics sample at the Department of Psychiatry, Trinity College Dublin, and through additional recruitment from local schools and child and adolescent mental health services. The diagnosis of ASD was established using two structured research diagnostic tools; the ADI-R (Autism Diagnostic Interview-Revised, (Lord *et al.*, 1994)) and the ADOS-G (Autism Diagnostic Observation Schedule-Generic, (Lord *et al.*, 2000)) and all participants met criteria for a diagnosis of ASD. Administrators of the ADI-R and ADOS-G were trained to and maintained reliability. Control participants were recruited from the community and were selected to match the participants with autism on age, handedness, gender, race and IQ (full-scale IQ was estimated based on 4 sub-scales of the WISC/WAIS (Wechsler Intelligence Scale for Children (WISC-IV UK), (Wechsler, 2004) and Wechsler Adult Intelligence Scale (WAIS-III), (Wechsler, 1997).

Exclusion criteria included any disorders associated with autism such as tuberous sclerosis or Fragile-X syndrome, current/past neurological or psychiatric conditions, serious head injuries, MR contraindications, below-average intelligence (full-scale IQ <80), current use of psychoactive medication and history of developmental delay (control exclusion only) or first-degree relatives with ASD (control exclusion only). The presence of neurological / psychiatric disorders was ruled out during a clinical assessment. The study was approved by the Irish Health Services Executive (HSE) Linn Dara / Beechpark Research Ethics committee and by the Ethics Committee of the School of Psychology in Trinity College Dublin.

Participants were provided with written information about the study and a recording of the noise of the scanner for familiarization. Pre-scanning visits to the MRI simulator were also offered. Written informed consent was obtained from parents (where appropriate) and participants prior to scanning. Before scanning, each participant was given full task instructions and trained with a set of practice trials for 15 minutes to become familiar with the stimuli and the button press responses. Participants were instructed to use mental imagery as a cognitive strategy for the rotation and transformation of the objects.

2.2. Experimental design - mental rotation task

Participants completed a mental rotation task that was used previously in studies investigating the neural correlates of mental rotation in normal healthy populations (Ecker *et al.*, 2006; Ecker *et al.*, 2008). The images presented in the task were based on Shepard and Metzler (Shepard and Metzler, 1971) stimuli (see Figure 1). These structures included five different three-dimensional objects and their five mirror images. Each object consisted of ten solid cubes attached face-to-face to form a rigid arm-like structure with exactly three right-angled elbows. Each structure was presented in shades of grey in front of a dark background. During the task, the stimuli were presented in pairs and displayed by a computer-controlled projector system on a screen. The 3D objects in each pair were either the same (Same Trial-type) or mirror images (Mirror Trial-type). In the Same Trial-type presentation, the two objects could be rotated into congruence with each other. In the presentation of a Mirror Trial-type, the two objects differed by a reflection (i.e. by being a mirror image of each other), as well as a rotation in either the x or z dimension and could not be rotated into congruence. As in the paradigm used by Richter et al (Richter *et al.*, 1997), object pairs were presented in four different experimental conditions according to the angular disparity between the objects: (1) 0° angular disparity [2 identical objects at the same orientation], (2) 20° angular disparity, (3) 60° angular disparity, and (4) 100° angular disparity.

Table 1: Demographics of study participants

	Control	ASD	p-value
Number	22	22	
Gender	Male (22)	Male (22)	
Age Mean (SD, Range)	17.51 (2.76, 13.6-21.3)	17.56 (2.91, 13.0-21.8)	0.76
Full scale IQ* Mean (SD, Range)	110.50 (16.97, 84-147)	105.95 (13.46, 84-127)	0.25
Verbal IQ^ Mean (SD, Range)	117.60 (12.69, 93-134)	105.60 (18.48, 79-134)	0.11
Performance IQ- Mean (SD, Range)	120.80 (26.39, 91-155)	124.35 (17.46, 99-155)	0.73
Handedness	Right (22)	Right (22)	
Medication	None	None	
Ethnicity	Irish (22)	Irish (22)	

*Full scale IQ was estimated based on four sub-scales of the WISC/WAIS (Wechsler Intelligence Scale for Children (WISC-III or IV UK), (Wechsler, 1991, 2004) and Wechsler Adult Intelligence Scale (WAIS-III), (Wechsler, 1997).

^Verbal IQ was estimated using the Information and Vocabulary subtests of the WISC III for n=10 participants with ASD and n=10 matched controls, using Sattler's method (Sattler, 1992). It was not possible to produce a Verbal IQ for n=12 participants with ASD and n=12 controls, as the WISC IV was used to estimate full scale IQ for these participants.

~ Performance IQ was estimated using the Picture Completion and Block Design subtests of the WISC III for n=10 participants with ASD and n=10 for controls, using Sattler's method (Sattler, 1992). It was not possible to produce a Performance IQ for n=12 participants with ASD and n=12 controls, as the WISC-IV was used to estimate full scale IQ for these participants.

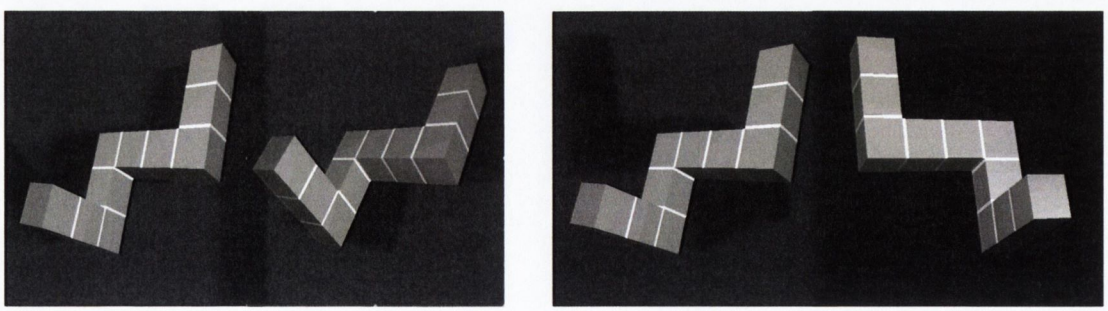


Figure 1: Example of task stimuli; Same pair, 20° rotation (left), Mirror pair, 20°rotation (right)

A total of 80 trials were presented in random order with respect to the same/mirror image condition and the angular disparity (0°, 20°, 60°, 100°). Each participant completed 20 trials of 0° same condition (where trials showed an identical pair of 3D images), and 20 trials of each of 20°, 60° and 100° where half of the trials per condition were the same pair, and the other half of the trials were a mirror pair. Unique stimuli pairs were used for the trials of each condition (i.e. no trials were repeated). Once the object pair appeared on the screen, subjects were asked to decide whether the objects were the same or mirror images. Subjects were instructed to respond as quickly as possible while keeping errors to a minimum, indicating their choice by pressing one of the two buttons on a keypad. The nature of the response (same or mirror image) and response times (RTs) were recorded. The task was presented in two runs of 40 trials per run.

2.3. Data acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a mirror that reflected the visual display, which was projected onto a panel placed behind the participants' head outside the magnet. The mirror was mounted on the head coil in the participant's line of vision. Each scanning sequence began with a reference scan to resolve sensitivity variations. A parallel Sensitivity Encoding (SENSE) approach (Pruessmann *et al.*, 1999) with a reduction factor of 2 was utilized for all T1-weighted image acquisitions. High resolution anatomical images were acquired using an MPRAGE sequence (180 oblique axial slices, field of view 230mm, thickness 0.9mm, voxel size 0.9mm×0.9mm×0.9mm, duration 5.43 minutes). These anatomical images allowed subsequent activation localization and spatial normalization. Functional data were collected using a T2* weighted echo-planar imaging sequence that acquired 39 non-contiguous (10% gap), 3.5mm axial slices covering the entire brain (TE 30msec, TR 2000msec, field of view 224mm, matrix size 64 x 64mm). The functional scans had a duration of 664sec per run.

2.4. Data analysis

2.4.1. Behavioural data analysis

Three-way mixed-factors repeated-measures ANOVAs were used to analyse the independent variables of Group (ASD, Control), Trial-type (Same, Mirror) and Degree of Rotation (DofR) (20°, 60°, 100°), for the dependent variables mean Response Time (RT) and Accuracy of responses (the 0° Same condition was excluded from these analyses as there was no corresponding 0° Mirror condition). The alpha level was set at 0.05 and Bonferroni corrections were used throughout the analyses.

2.4.2. fMRI data analysis

All analyses were conducted using Analysis of Functional Neuroimages software [AFNI, http://afni.nimh.nih.gov/afni] (Cox, 1996). After image reconstruction, time differences in slice acquisition were removed using a Fourier interpolation. The time series data were motion corrected using 3D volume registration (least squares alignment of three translational and three rotational parameters). Individual images were excluded from the analyses if there was greater than 0.9mm translational movement or greater than 0.9 degrees rotational movement. Each time series was then temporally filtered with a high-pass filter of 0.036Hz (to remove drift in signal intensity). An edge detection algorithm was used to remove activations from outside the brain and functional and anatomical images were coregistered.

For every participant, a regressor was generated for each DofR and Trial-type (0° Same, 20° Same, 20° Mirror, 60° Same, 60° Mirror, 100° Same, 100° Mirror) based on the convolution of a boxcar function corresponding to the trials of that condition with a gamma-variate function (Cox, 1996). A multiple regression analysis was then carried out comprising seven task-related regressors (one for each experimental condition) and the motion-corrected time-series regressors to accommodate nuisance variance. The beta-weights were converted to a percentage-change score (i.e., a percentage change in activation over baseline) and these maps were warped onto the Talairach space (Talairach and Tournoux, 1988), resampled to 1mm cubic voxels, and spatially blurred with a 3-mm isotropic rms Gaussian kernel filter. An edge detection algorithm using a mask

generated from the normalized, skull-stripped anatomical data was then used to remove activation outside the brain.

Group activation maps for each condition for both groups were determined with one sample t-tests against the null hypothesis of no activation change. Significant voxels passed a voxelwise statistical threshold ($t = 2.96$, $P \leq 0.005$) and were required to be part of a larger 286 µL cluster of contiguous significant voxels. This cluster size was determined through a Monte Carlo simulation and resulted in less than a 5% probability of a cluster surviving due to chance.

To investigate the neuroimaging correlates of between-group differences and between Trial-type differences, a voxel-wise analysis was performed using a two way repeated measures ANOVA [Group (ASD/Control) x Trial-type (Same/Mirror)] with activations averaged over the 20˚, 60˚and 100˚ conditions. This analysis was chosen as preliminary behavioural analysis showed no between-group differences in RT at each degree of rotation, but did reveal an interesting dissociation between groups in RTs for the Same and Mirror Trial-type. A primary aim of this study was to investigate brain activity during mental rotation in order to increase understanding of the pathophysiology of atypical visuospatial processing. Given the novel between-group difference in RTs on Same and Mirror Trial-type, it was felt that an examination of the neural correlates of these factors was important as it would provide a unique insight into possible neurobiological underpinnings of atypical visuospatial processing in ASD. Thresholding was carried out using criteria as described above for the group activation maps.

3. Results

3.1. Behavioural results

3.1.1. Mean response time

Overview

The Control group performed the mental rotation of the Same Trial-type significantly more quickly than that of the Mirror Trial-type at all three degrees of rotation. This performance of the Control group was in strong

contrast with that of the ASD group, which showed a consistent mean response time (RT) in mental rotation of both the Same and Mirror configurations, at all three degrees of rotation (Figure 2). In other words, the Mirror Trial-type did not seem to slow the rate of mental rotation of the ASD group to the same extent as shown by the Control group, when compared with the speed of rotation of the Same configuration. The mean RTs for the two groups for both Trial-types increased as the degrees of rotation increased: both groups were able to rotate the figures most quickly at 20°, then 60° and most slowly at 100° rotation. In terms of the speed of mental rotation at each of the three degrees of rotation, the ASD group performed in a similar manner to the Control group.

There was a significant 3-way (Group x Trial-type x Degree of Rotation (DofR)) interaction for mean response time (RT) [$F(2,84)=4.419$, $p<0.015$, $\eta p2=0.095$], which was first broken down by Group (Figure 2). Two-way ANOVAs were run separately for each Group. For controls, this analysis revealed a significant DofR x Trial-type interaction [$F(2,42)=5.648$, $p<0.007$, $\eta p2=0.212$]. Pairwise comparisons revealed that controls performed the Same Trial-type significantly faster than the Mirror Trial-type at all three degrees of rotation (20° $p<0.001$, 60° $p<0.001$, 100° $p<0.031$). Pairwise comparisons for DofR (20° v 60°, 20°v 100°, 60°v 100°) were all significant for both Same and Mirror Trial-types (all $p<0.001$) indicating that controls slowed as task difficulty increased with increasing DofR. For the ASD group there was no interaction between DofR and Trial-type and no main effect of Trial-type (both $p>0.050$); indicating that the ASD group performed the Same and Mirror Trial-types with similar mean RTs at 20°, 60° and 100°. As with the control group, there was a significant main effect of DofR [$F(2, 42)=128.17$, $p<0.01$, $\eta p2=0.859$] – the ASD group also slowed in response time as DofR increased.

To specifically investigate if there were between-group differences in mean RT, the 3-way interaction was secondly broken down by DofR. Two-way ANOVAs were run separately for each DofR. At 20°, this analysis revealed a significant DofR x Group interaction [$F(1,42)=6.910$, $p<0.012$, $\eta p2=0.141$]. The control group performed the Same condition significantly more quickly

than the Mirror condition ($p<0.0001$). In contrast, there was no significant difference in the ASD group for the Same and Mirror conditions ($p>0.124$). Thus the interaction was driven by the slowing of controls on the Mirror condition. There were no significant differences in mean RT between ASD and control groups on either 20° Same ($p>0.336$) or 20° Mirror ($p>0.135$) Trial-types. At 60°, there was a significant DofR x Group interaction [$F(1,42)=10.296$, $p<0.003$, $\eta p2=0.197$]. As at 20°, the control group performed the Same condition significantly more quickly than the Mirror condition ($p<0.0001$) and again, there was no significant difference in performance by the ASD group for the Same and Mirror conditions ($p>0.353$). Thus, again, the interaction was driven by the slowing of controls on the 60° Mirror condition. Both groups performed the 60°conditions at similar speeds (Same Trial-type $p>0.245$, Mirror Trial-type $p>0.149$). At 100°, there was no DofR x Group interaction and no Group main effect (both $p>0.050$). There was a significant main effect of Trial-type [$F(1,42)=11.637$, $p<0.001$, $\eta p2=0.217$] with both groups slowing on the Mirror Trial-type.

Mean Response Time (seconds)		ASD	Control
	20° Same	2.60 (SD 0.83)	2.38 (SD 0.74)
	20° Mirror	2.91 (SD 0.95)	3.50 (SD 1.19)
	60° Same	3.93 (SD 1.36)	3.49 (SD 0.98)
	60° Mirror	4.12 (SD 1.06)	4.52 (SD 1.10)
	100° Same	4.61 (SD 1.15)	5.13 (SD 1.30)
	100° Mirror	5.03 (SD 0.86)	5.54 (SD 1.06)
Accuracy (%)			
	20° Same	89.3 (SD 3.6)	90.9 (SD 0.0)
	20° Mirror	92.4 (SD 9.3)	90.9 (SD 10.6)
	60° Same	94.5 (SD 9.6)	94.1 (SD 5.9)
	60° Mirror	91.8 (SD 10.0)	90.9 (SD 8.7)
	100° Same	88.4 (SD 13.1)	79.8 (SD 16.3)
	100° Mirror	84.3 (SD 10.2)	88.0 (SD 10.3)

Table 2: Mean response time and accuracy data

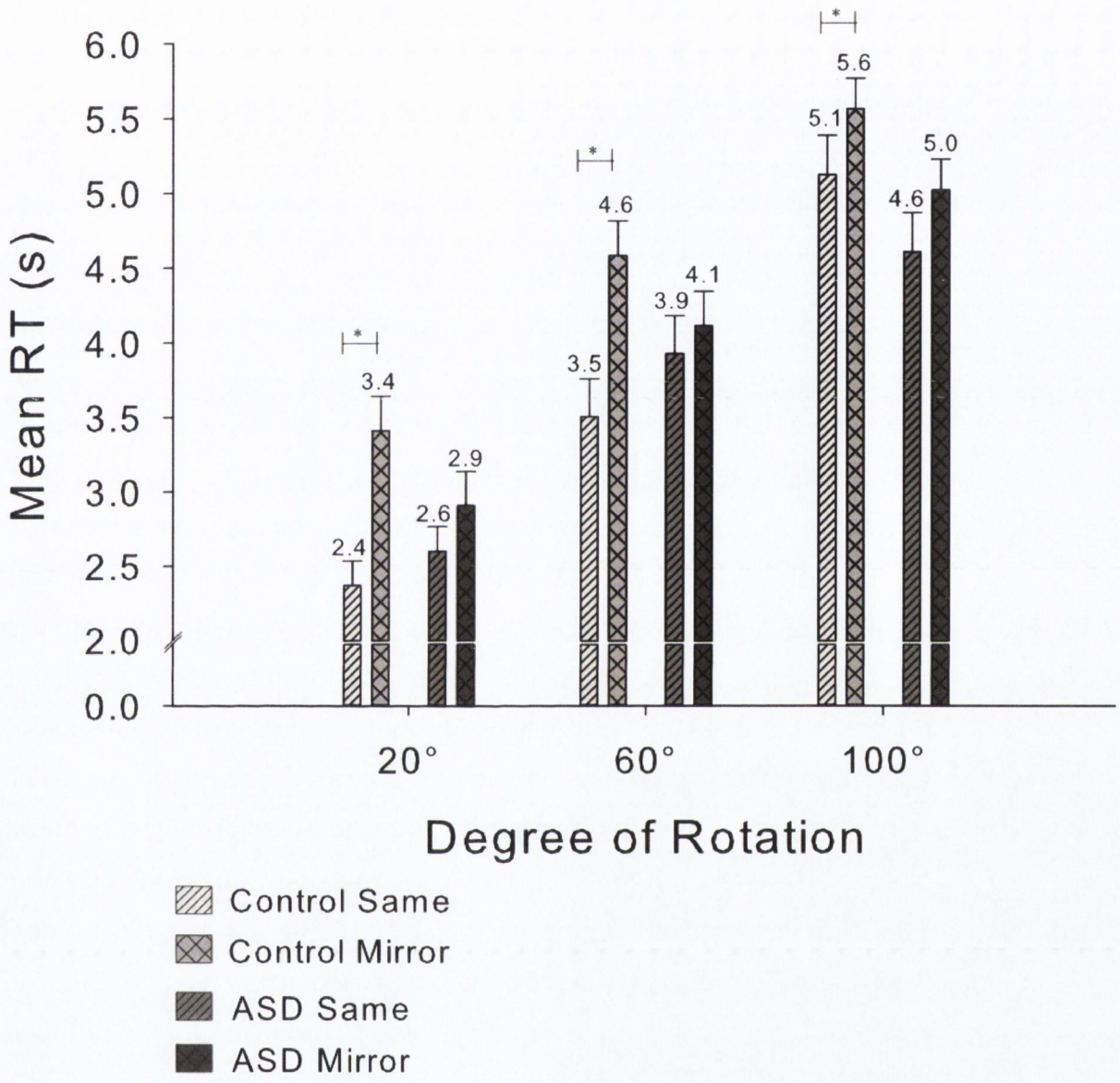


Figure 2: Mean Response Time (RT) for the Control and ASD groups for the Same and Mirror figures at 20, 60 and 100 degrees of rotation.

3.1.2. Accuracy

Overview

ASD and Control groups showed similar accuracy for all degrees of rotation on Same and Mirror trials. The Control group showed a significant deterioration in accuracy at 100° rotation in the Same condition and performed more poorly on this condition than the 100° Mirror condition. The ASD group showed consistency in accuracy between degrees of rotation and between Same and Mirror conditions.

A three-way (Group x Trial-type x DofR) ANOVA revealed a significant 3-way interaction [$F(2, 84)=5.867$, $p<0.004$, $\eta p2=0.123$]. Given the apparent locus of this 3-way interaction at 100° (see Figure 3), the between-group differences in accuracy were investigated by breaking down the 3-way interaction by DofR. For both the 20° and 60° conditions there was no significant interaction between Group and Trial-type, nor any significant main effects, indicating that the two groups performed the task with similar accuracy rates. At 100° there was a significant Group x Trial-type interaction [$F(1,42)=6.576$, $p<0.014$, $\eta p2=0.135$]. Controls were significantly less accurate on the Same Trial-type compared with the Mirror Trial-type at 100° ($p<0.014$). In contrast, the ASD group showed no difference in accuracy between Trial-types ($p>0.290$). The two groups performed the 100°Mirror Trial-type with similar levels of accuracy ($p>0.234$). On the 100°Same Trial-type, the ASD group was significantly more accurate than controls ($p<0.039$). The 3-way interaction appeared to be driven by the reduced level of accuracy by the Control group on the 100°Same Trial-type.

To summarise the behavioural results, there was no significant between-group differences in terms of the speed of mental rotation at each of the three DofR. There was a dissociation between the ASD and control groups in that the controls slowed significantly on Mirror Trial-type relative to Same Trial-type whereas the ASD group did not slow, but showed similar mean response times for Same and Mirror trials at all three degrees of rotation. The ASD group was significantly more accurate than controls on 100° Same trials.

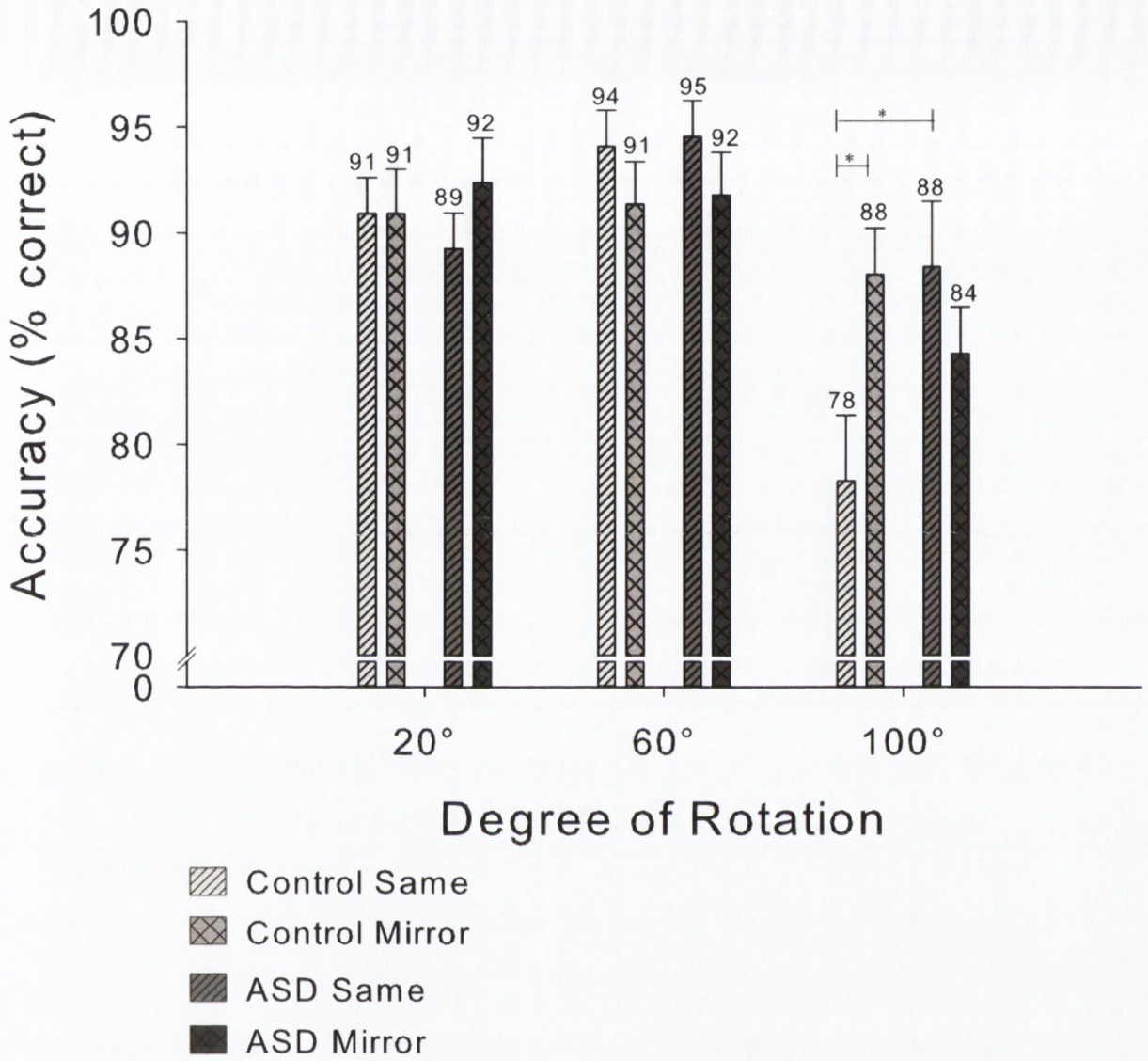


Figure 3: Accuracy data for the Control and ASD groups for the Same and Mirror figures at 20, 60 and 100 degrees of rotation.

3.2. Functional MRI results

3.2.1. Group activation maps

The group activation maps for both the Control and ASD groups revealed activation in a wide network of brain regions that have been consistently reported in previous imaging studies on mental rotation (Zacks, 2007). Activated regions included frontal (e.g. precentral cortex, BA 44 and BA 45), parietal (e.g. superior parietal cortex and intraparietal sulcus extending into the transverse occipital sulcus including BA 7, 19, 39 and 40), inferotemporal (e.g. posterior inferior temporal cortex) and occipital regions (e.g. early visual areas BA17, and dorsal extrastriate cortex BA18, 19) (see Figures 4 and 5).

3.2.2. Main effect of Group

The ASD group, relative to the Control group, showed decreased activation in the right hemisphere in frontal (superior and inferior frontal gyrus), temporal (fusiform gyrus), occipital (lingual gyrus, cuneus) and subcortical (caudate) regions and in the left hemisphere in frontal (inferior frontal gyrus), occipital (inferior occipital gyrus), and cerebellar (inferior semilunar lobule, cerebellar tonsil and tuber) regions (see Table 3 and Figure 6). There was no significant Group by Trial-type interaction.

3.2.3. Main effect of Trial-type

A significant main effect for Trial-type revealed that the Mirror Trial-type produced greater activity compared with the Same Trial-type, in a number of regions including right hemisphere frontal (inferior frontal gyrus, precentral gyrus), parietal (inferior and superior parietal lobule, precentral gyrus), temporal (middle temporal gyrus), occipital (cuneus, inferior occipital gyrus) and subcortical (caudate) regions and left hemisphere frontal (superior, middle and inferior frontal gyrus, precentral gyrus), parietal (inferior parietal, precuneus), temporal (cingulate gyrus, fusiform gyrus), cerebellar (declive) and subcortical (thalamus and caudate) regions (see Table 3).

			Location	BA	X	Y	Z	Voxels	Effect
Main effect of Group									
	Frontal	L	Inferior Frontal Gyrus	47	35.4	-27.2	-12.2	354	C > ASD
		R	Inferior Frontal Gyrus	47	-41.9	-21.2	-2.4	1067	C > ASD
		R	Superior Frontal Gyrus	8	-0.7	-22.6	47.9	1254	C > ASD
	Temporal	R	Fusiform Gyrus	37	-55.6	51.1	-19.2	573	C > ASD
	Occipital	L	Inferior Occipital Gyrus	19	42	73.3	-1.5	1770	C > ASD
		R	Lingual Gyrus	19	-34.4	73	-9.5	616	C > ASD
		R	Cuneus	18	-11.5	75.3	6.2	505	C > ASD
	Cerebellum	L	Inferior semilunar lobule		30.9	64.8	-45.4	1506	C > ASD
		L	Cerebellar Tonsil		35.1	34.4	-43.6	814	C > ASD
		L	Tuber		49.9	49.3	-26.4	603	C > ASD
	Subcortical	R	Caudate		-15.4	-0.9	17.6	553	C > ASD
Main effect of Trial type									
	Frontal	L	Superior Frontal Gyrus	6	1.1	-9.9	49.3	4676	M > S
		L	Middle Frontal Gyrus	6	26.3	8.2	56.9	2828	M > S
		L	Middle Frontal Gyrus	46	40.7	-37.3	22.7	2449	M > S
		L	Cingulate Gyrus	24	4	-14.3	26.1	297	M > S
		L	Precentral Gyrus	6	43.6	-3.6	37.3	1935	M > S
		R	Precentral Gyrus	6	-26.6	12.1	53.6	1347	M > S
		R	Precentral Gyrus	6	-39.8	0.4	37.4	721	M > S
		R	Inferior Frontal Gyrus	13	-32.3	-28	12.2	1036	M > S
	Parietal	L	Precuneus	7	25.2	66.8	34.1	3037	M > S
		L	Precuneus	7	11.6	59.4	47.9	428	M > S
		L	Inferior Parietal Lobule	7	33.7	51.1	49	1518	M > S
		R	Inferior Parietal Lobule	40	13.8	18.2	17.4	1488	M > S
		R	Superior Parietal Lobule	7	-26.1	57.3	47.4	1091	M > S
	Temporal	R	Middle Temporal Gyrus	19	-40.8	78.8	23.6	323	M > S
	Occipital	R	Cuneus	18	-19	79.3	29.2	633	M > S
		R	Inferior Occipital Gyrus	19	-40.6	74.3	-2.1	517	M > S
	Cerebellum	L	Cerebellar Vermis		0.3	37.6	-33.9	2152	M > S
		L	Declive + Fusiform Gyrus		24	64.3	-11.1	766	M > S

Table 3: Group and Trial type effects from the voxelwise ANOVA (C: Control group, ASD: ASD group, M: Mirror Trial Type, S: Same Trial Type).

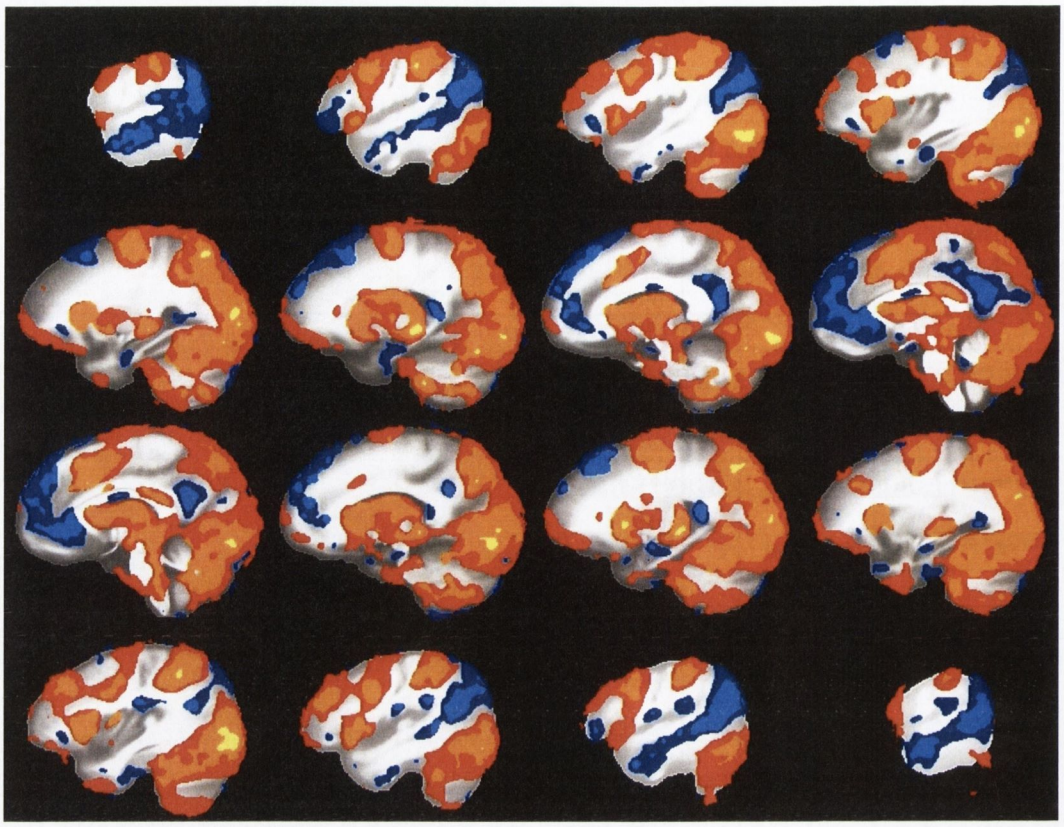


Figure 4: Control group activation map during the mental rotation task

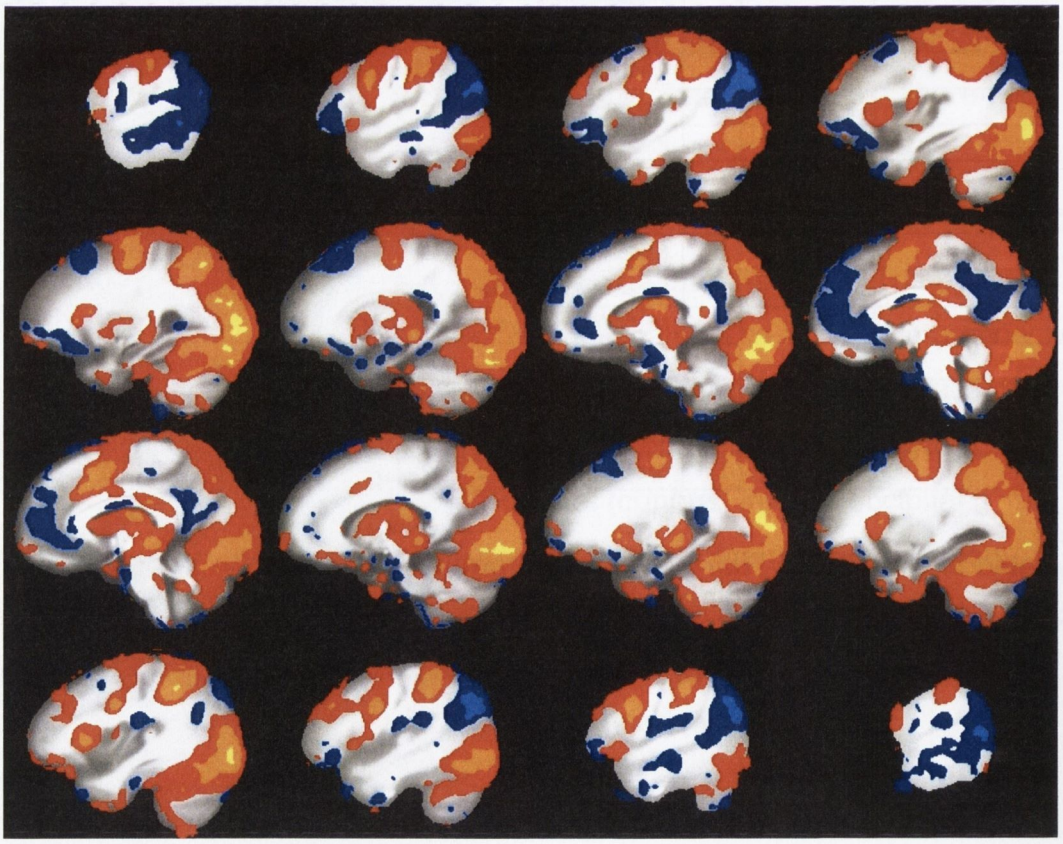


Figure 5: ASD group activation map during the mental rotation task

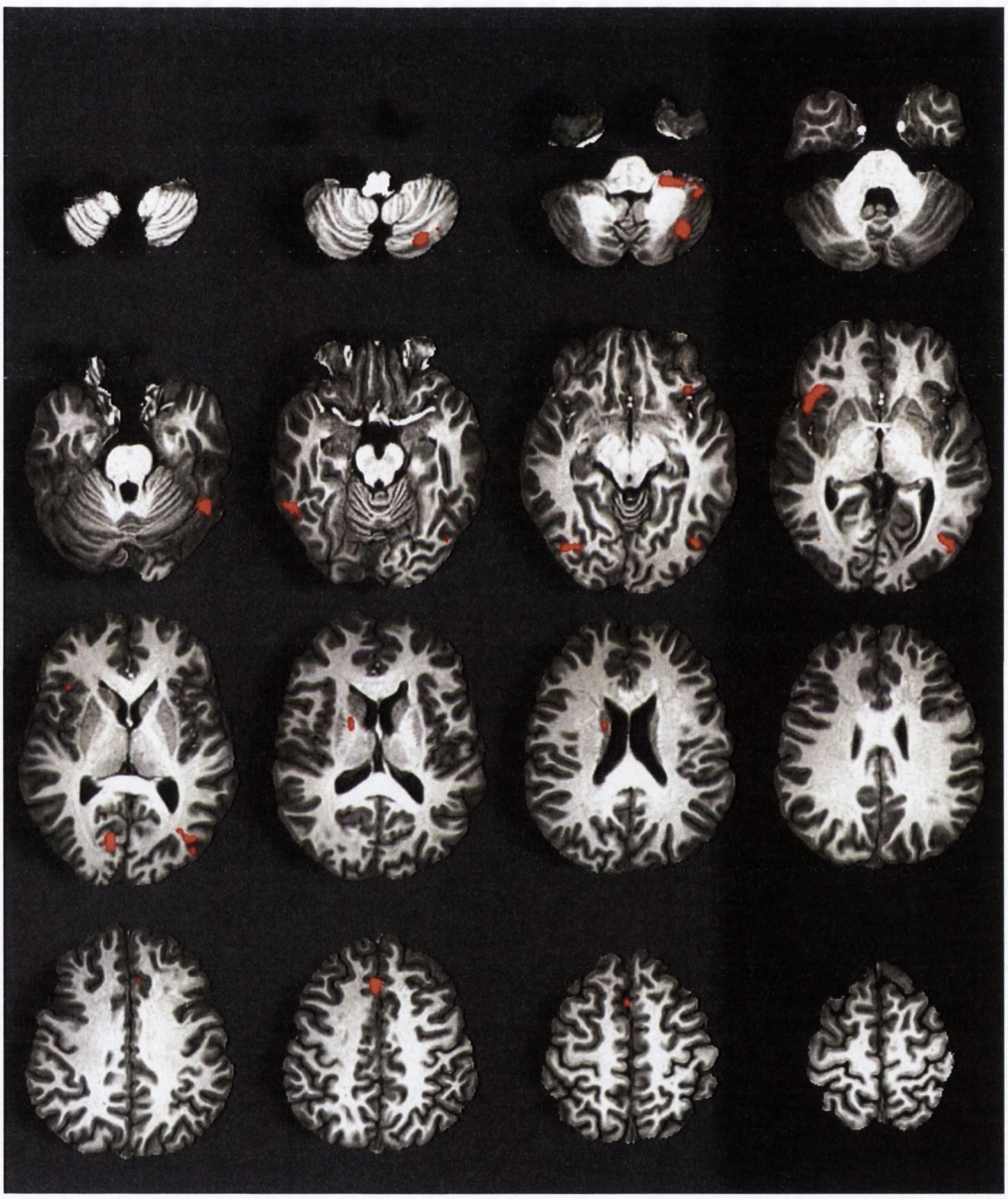


Figure 6: Group Main Effect - Axial sections showing regions of significant activation differences (red) between Control and ASD groups during mental rotation ($p<0.05$, corrected). All regions showed greater activation in the Control group.

4. Discussion

The aim of this study was to investigate the neurocognitive basis of mental rotation differences between individuals with ASD and typically developing controls in order to increase understanding of the neurobiological underpinnings of atypical visuospatial processing in the disorder.

4.1. Behavioural results

Behavioural analysis revealed a striking and previously unreported dissociation between the ASD and Control groups in response times for Same and Mirror Trial-types. Controls took significantly longer on Mirror Trial-type than on Same Trial-type, whereas the ASD group performed the two Trial-types at the same speed. Although mental rotation is an extensively studied cognitive function, there is surprisingly little research investigating the differences between Same and Mirror Trial-types. It seems that more rotation is involved during the processing of Mirror Trial-type (Hamm *et al.*, 2004) and this condition appeared to provide a more cognitively demanding mental rotation task for the Control group only, perhaps suggesting superior mental rotation ability in the ASD group (as has been reported in a small number of publications (e.g. (Falter *et al.*, 2008; Soulieres *et al.*, 2011). Soulieres et al, 2011 suggest that enhanced mental rotation abilities in ASD may result from superior 'veridical mapping', a low-level visual cortical process referring to the detection of similarity among visual patterns, that contributes to a low-level visual processing bias. Cognitive mechanisms in veridical mapping involve a feed-forward, bottom-up process that is less reliant on global top-down, higher cognitive processes. This theory offers a cognitive explanation for our behavioural findings in that a parallel "veridical" mapping of corresponding components of the two stimuli is likely to make the mirror and same task conditions similarly difficult for the ASD group. Another recent study investigating visual grouping in autism reported that individuals with autism are significantly better at detecting mirror symmetry than non-autistic individuals (Perreault *et al.*, 2011). The authors suggest that this superior performance may result from enhanced pattern detection in autism (Mottron *et al.*, 2009; Soulieres *et al.*, 2011) and hypothesise that there may be an atypical visuospatial

processing mechanism in autism involving parallel access to local and global information (Perreault *et al.*, 2011). The behavioural results reported here are in keeping with this finding of superior detection of mirror symmetry in autism and may implicate the use of different visuospatial processing mechanisms by the two groups during mental rotation. It would appear that averaging response times across Same and Mirror Trial-types (which has been reported in a number of previous studies on mental rotation) may have masked an important between-group difference in mental rotation performance on these two Trial-types.

4.2. fMRI results

4.2.1. Introduction

Functional MRI results revealed that in comparison with the Control group, the ASD group had significantly reduced brain activity in multiple regions normally involved in mental rotation including frontal (BA 47, BA 8), occipito-temporal (BA 37), occipital (BA 18, 19), striatal and cerebellar regions. Previous fMRI studies have reported similar findings of successful visuospatial task performance despite an overall reduction in cortical activation (Silk *et al.*, 2006; Lee *et al.*, 2007). In these studies, no specific explanation was offered for how successful task performance was achieved with such reduced cortical involvement, but the results would suggest that visuospatial processing in ASD is accomplished in an atypical way. In addition, both groups showed significantly greater activity during Mirror trials relative to Same trials. This may reflect the additional rotation required during these trials and is consistent with the theory that Mirror trials present a more demanding mental rotation task (Hamm *et al.*, 2004).

The following discussion provides a review of each of the brain regions in which there were between-group differences in activation during mental rotation. The postulated roles of each region in mental rotation are outlined and possible reasons for the between-group differences in brain activation are considered. Links between known brain structural abnormalities in autism and the functional activation differences reported in this study are investigated.

4.2.2. Frontal cortex

Significant activation in the left and right prefrontal cortex occurred in both ASD and control groups. There was greater activation in the control group in bilateral inferior frontal gyrus and right superior frontal gyrus during mental rotation. These areas are discussed below.

Inferior prefrontal cortex

During the task, activation was observed in the inferior frontal gyrus in regions corresponding to BA 44, 45 and 47. Relative to controls, there was significantly less activation in the ASD group bilaterally in BA 47.

A recent metanalysis of fMRI studies of mental rotation reported consistent activation of regions BA 44 and BA 45 in the inferior prefrontal cortex (Zacks, 2007). In this study, bilateral activation of these areas is seen in both groups. These regions have not only been associated with speech production, but also with motor control and imitation (Rizzolatti *et al.*, 1996). It has been suggested that activation of these regions during mental rotation may reflect the use of motor simulation (Zacks, 2007).

The area of difference between the two groups in the inferior frontal gyrus had a central focus point in BA 47. Activation of this region has previously been described during mental rotation (Podzebenko *et al.*, 2002). BA 47 is a cortical association area that is thought to participate in prefrontal cortical networks that govern executive functions. Studies have reported that it is involved in response inhibition (Vollm *et al.*, 2006), attribution of intention to others / theory of mind (Brunet *et al.*, 2000), deductive reasoning (Goel *et al.*, 1997) and decision making (Rogers *et al.*, 1999). BA 47 is also thought to play an important role in higher processing of language – for example in semantic processing (De Carli *et al.*, 2007), phonological processing (McDermott *et al.*, 2003; De Carli *et al.*, 2007) and semantic encoding (Demb *et al.*, 1995) among others.

The reduction of prefrontal activation in this study may be secondary to disruption of long-range fronto-posterior neural connectivity, a finding which has been widely reported in the literature in ASD [e.g. (Just *et al.*, 2004) and see (Schipul *et al.*, 2011) for review]. Two previous neuroimaging studies

using visuospatial paradigms have reported reduced prefrontal cortical activation associated with reduced long-range fronto-posterior functional connectivity (Kana *et al.*, 2006; Damarla *et al.*, 2010). Alternatively, it is possible that the ASD group required less top-down executive control, due to enhanced low-level perceptual processing at the level of the visual cortex (Mottron *et al.*, 2006). Given its role in language processing, reduction of activation in BA47 may represent a reduced reliance of the ASD group on verbal/linguistic strategies to perform mental rotation. There is accumulating evidence to suggest that individuals with ASD have a greater predisposition to visual thinking and that they show an increased reliance on visuospatial processing strategies over linguistic strategies to solve problems (Toichi and Kamio, 2001; Kana *et al.*, 2006; Sahyoun *et al.*, 2010). Preferential recruitment of visuospatial over linguistic processing regions could be secondary to disrupted connectivity between frontal language and posterior regions, or to hyperspecialisation of early visual regions. Functional connectivity during mental rotation, however, has never been investigated in individuals with ASD.

A number of studies have reported a variety of structural abnormalities of the orbitofrontal cortex (OFC) (which includes BA 47) in ASD. Volumetric studies have reported significant differences in grey matter volume in the OFC in ASD (Hardan *et al.*, 2006; Bonilha *et al.*, 2008). Studies investigating cortical thickness have also reported abnormalities of the OFC with findings of increased (Ecker *et al.*, 2010) or reduced cortical thickness (Chung *et al.*, 2005; Jiao *et al.*, 2010). Diffusion tensor imaging studies have revealed white matter abnormalities in this region with reduction of fractional anisotropy in OFC (Barnea-Goraly *et al.*, 2004). A reduction in integrity of white matter in this region could contribute to the reduced fronto-posterior functional connectivity that has been widely reported in the literature – this is discussed in detail in chapter 1, section 2.1.

Superior frontal gyrus - BA 8

There was widespread superior and middle prefrontal cortex activation in both groups during the mental rotation task. Activity was seen bilaterally in the superior frontal gyrus in regions corresponding to BA 8 and in

superior/middle frontal gyrus in BA 9 and 46. These regions have all previously been reported in studies of mental rotation (e.g. (Cohen *et al.*, 1996; Schoning *et al.*, 2007).

In this study a region of right BA 8 showed a significant between-group difference in activation with reduced activity in the ASD group. BA 8 is located in the dorsolateral prefrontal cortex (DLPFC). It has traditionally been regarded as the Frontal Eye Field, a region involved in the control of eye movements (Anderson *et al.*, 1994). Neuroimaging research has shown BA 8 to be involved in a diverse range of other functions including executive functions (Sarazin *et al.*, 1998; Kubler *et al.*, 2006), working memory (Rama *et al.*, 2001; Babiloni *et al.*, 2005), inductive reasoning (Goel *et al.*, 1997) and theory of mind (Fletcher *et al.*, 1995). Therefore the activation of BA 8 during mental rotation may be associated with the considerable saccadic scanning that is known to occur during mental rotation (Just and Carpenter, 1985) but it may also reflect the demands of the task on working memory, executive control and inductive reasoning.

If there is enhanced perceptual functioning at an early visual cortical level in ASD (e.g. (Mottron *et al.*, 2006; Soulieres *et al.*, 2011), the reduction in activity of BA 8 may reflect a reduced need for this group to use horizontal saccades to repeatedly compare the two images presented in the task. Alternatively, as discussed above in relation to BA 47, it is also possible that the reduced activation in this frontal region in ASD indicates a reduction of top-down executive processing which may be a consequence of impaired fronto-posterior neural connections.

Interestingly, a number of studies have identified structural abnormalities of BA 8 in autism, which could all contribute to a disruption of fronto-posterior connectivity in the disorder. Increased grey matter of right hemisphere BA 8 (Bonilha *et al.*, 2008), reduced cortical thickness bilaterally in BA 8 (Hadjikhani *et al.*, 2006) and reduced fractional anisotropy in left DLPFC (Noriuchi *et al.*, 2010) have all been reported.

Precentral cortex (BA 6 specifically motor regions)

During mental rotation, extensive bilateral activation in the precentral cortex was observed in both the ASD and Control groups. There were no between-group differences in activation in these regions.

Since the first neuroimaging studies of mental rotation, activation in motor regions has been reported (Cohen *et al.*, 1996) indicating that motor strategies are required for successful mental rotation. A recent metanalysis of studies of mental rotation reports bilateral activation in the precentral sulcus (Zacks, 2007). This region is typically associated with motor execution and motor planning. During a mental rotation task, the activation in motor regions could simply be due to the task requirement to plan and perform a manual motor response (e.g. button press). It has been suggested, however, that the motor activation that occurs during mental rotation tasks is not solely associated with planning and execution of a motor response, but that it may also reflect the use of motor simulation to solve the mental rotation task (Michelon *et al.*, 2006). From the results of their metanalysis, Zacks et al suggest that activation in the superior medial portion of the precentral sulcus (a region that corrresponds to the supplementary motor area - SMA) is associated with motor simulation (mental rotation) whereas activation in the infero-lateral precentral sulcus is associated with planning and execution of the manual motor response to the task. In this study, there was extensive activation in both these regions of the precentral sulcus during the task in both the ASD and Control groups.

4.2.3. Occipital cortex

Significant visual system activation was observed in early visual areas (BA17), in the medial-occpital/inferior temporal complex (BA19/37) and in dorsal extrastriate visual areas (BA18/19). Relative to controls, the ASD group showed a significant reduction in activation in the right cuneus (BA17/18), in bilateral BA19, and in right BA37.

Right cuneus (BA 17/18 - Early visual cortex)

Activation of the early visual cortex has been widely reported during mental rotation (Vingerhoets *et al.*, 2001; Ecker *et al.*, 2006) and see (Podzebenko

et al., 2002). In this study, controls showed greater activity in the right cuneus (BA 17/18). This region is involved in basic visual processing – for example in detecting light intensity, colour, patterns and contours. There is also some evidence to suggest that the cuneus is also activated during mental imagery tasks (Buchel *et al.*, 1998). The difference in right cuneus activation could represent a difference in basic visual processing, or possibly in how mental imagery is used to imagine the 3D shapes in different rotational positions.

Dorsal extrastriate cortex

BA 18 and 19 comprise the extrastriate cortex. This region is a visual association area, with roles in attention, shape recognition and feature extraction. BA 19 surrounds BA 18, and is located in the superior and lateral occipital gyrus, the cuneus and parts of the lingual gyrus. Involvement of extrastriate visual regions during mental rotation has been widely reported in previous studies [see (Podzebenko *et al.*, 2002) for discussion]. The ASD group showed significant reduction of activation bilaterally in a large region of BA18/19.

Temporal-occipital cortex

During mental rotation, a number of studies have reported activation of a particular region of the visual cortex located in the temporo-occipital cortex in BA 19/37 – the middle temporal MT/V5 (Cohen *et al.*, 1996; Pegna *et al.*, 1997). This motion-sensitive MT/V5 complex (BA 19/37) is selectively activated by rotary motion (V5A) (Haug *et al.*, 1998) and an area adjoining V5 is activated by illusory motion (Zeki *et al.*, 1993). In our study, this area was activated in both groups, but the control group showed significantly greater activity in this region of left V5. Controls also showed greater activity in right BA37.

The greater control activity in the three visual cortical regions discussed above may reflect between-group differences in the mechanisms of visual processing of the task stimuli. If there was a low-level processing bias in the early visual cortex during mental rotation (Mottron *et al.*, 2006), one might expect to see a relative increase in activation of this region compared with

controls (Samson *et al.*, 2011). Conversely, a significantly reduced visual cortical activity in the ASD group was observed in this study, a finding in keeping with results from two other neuroimaging studies investigating visuospatial processing in ASD (Silk *et al.*, 2006; Lee *et al.*, 2007). Hypothesised causes of enhanced low-level visual processing in autism include hyperspecialisation of low-level processing areas secondary to short-range overconnectivity [e.g. (Just *et al.*, 2004; Wass, 2011) for review)] or excess lateral inhibition in the visual cortex (Bertone *et al.*, 2005; Vandenbroucke *et al.*, 2008). Lateral inhibition is a process that plays a crucial role in determining object orientation. If this is the case, then the reduced activity in the ASD group could be interpreted to represent more efficient neural processing at a low level in the early visual cortex.

Structural abnormalities may underpin the observed between-group differences in functional activation in early visual, extrastriate and occipito-temporal regions. Increased grey matter volume in bilateral BA 19, bilateral BA37 and right BA 18 and reduced white matter volume in bilateral cuneus and left BA37 has been reported (Bonilha *et al.*, 2008). Also, increased cortical thickness in ASD has been reported in left BA17, right BA18/19 and left BA19 (Hyde *et al.*, 2010). Again, it seems that there may be a link between brain structural and functional abnormalities.

4.2.4. Cerebellum

Cerebellar activity during mental rotation has been reported previously in the literature (Dupont *et al.*, 1993; Bonda *et al.*, 1995; Schiltz *et al.*, 1999; Podzebenko *et al.*, 2002; Zacks *et al.*, 2002). In this study widespread cerebellar activation was observed in both ASD and control groups during the mental rotation task. A significant reduction in activity in the ASD group relative to controls was observed in large cerebellar regions in the left hemisphere including the inferior semilunar lobule, cerebellar tonsil, tuber and culmen.

Authors of a number of previous studies have expressed surprise at the involvement of the cerebellum during mental rotation, perhaps as the cerebellum has typically been regarded as being involved exclusively in motor activities. It is now known that the cerebellum has connections with

cortical regions involved in emotional and cognitive functions and it is thought that it may play a similar role in these domains as in the regulation of motor function (Stanfield *et al.*, 2008). A study specifically investigating the role of the cerebellum in mental rotation suggests that the cerebellum is engaged immediately in the sensory and cognitive processing of the visual images that are required to determine the correct task response (Houck *et al.*, 2007). Cerebellar involvement in mental rotation is perhaps not at all surprising given that it is reciprocally connected to the parietal cortex, a region that plays a crucial role in mental rotation. Parietal projections reach the cerebellum via the pontine nuclei and projections from the dentate nucleus of the cerebellum reach the parietal (and prefrontal) cortices (Clower *et al.*, 2001; Middleton and Strick, 2001; Allen *et al.*, 2005). Allen et al, 2005 demonstrated strong functional connectivity between BA 7 of the parietal cortex and the dentate nucleus of the cerebellum (Allen *et al.*, 2005). In the present study, activation of BA 7 in both ASD and control groups was observed.

The cerebellum is a particularly interesting region in autism as anatomical abnormalities of this region have been reported consistently. Reduced numbers of Purkinje cells (GABAergic neurons located in the cerebellar cortex) have been reported in every post-mortem brain study in autism making this "the most reproducible pathological observation in the autopsied autistic brain" (Bauman and Kemper, 2005). In addition, a metanalysis of volumetric studies reveals that cerebellar size is increased in autism (Stanfield *et al.*, 2008) and recent DTI studies have revealed reduced fractional anisotropy in the middle cerebellar peduncle (Shukla *et al.*, 2010), short intracerebellar fibres, right superior cerebellar (output) peduncle (Catani *et al.*, 2008) and other diffusion changes (increased axial diffusivity) in the cerebellar vermis (Noriuchi *et al.*, 2010). Allen and Courchesne suggest that a deficit of cerebellar regulatory role on cognition may explain the difficulties people with ASD have in switching attention and in interpreting social situations (Allen *et al.*, 2004). The differences in cerebellar activity between controls and ASD group in this study may be linked to these anatomical abnormalities.

4.2.5. Caudate

Activation of the caudate nucleus has been reported during mental rotation (Alivisatos and Petrides, 1997; Silk *et al.*, 2006). In this study, bilateral caudate activation occurred in the ASD and control groups, with significantly greater and more extensive activation in the right caudate in controls.

The caudate is likely to play a critical role in mental rotation. Impairments in mental rotation have been reported in conditions characterised by caudate pathology (Mohr *et al.*, 1991; Lee *et al.*, 1998; Harris *et al.*, 2002). In addition, the basal ganglia (containing the caudate nucleus) receive extensive projections from the parietal lobe (a region considered crucial for successful mental rotation) (Selemon and Goldman-Rakic, 1988; Cavada and Goldman-Rakic, 1991; Yeterian and Pandya, 1993) and have strong connections to the motor cortex (another region implicated in mental rotation). The basal ganglia are thus in an ideal location to integrate information about visuospatial and motor components of mental rotation processes. Alivisatos and Petrides suggest that the head of the caudate nucleus may be involved in the "purely cognitive motion of stimuli in space" (Alivisatos and Petrides, 1997). The authors cite evidence that a class of caudate neurons in the monkey has a preferential relationship to the internal generation of movement (Romo *et al.*, 1992). This could suggest that caudate activation reflects the imagined rotation that is required for successful mental rotation. Caudate involvement during mental rotation may also reflect the action selection or motor response preparation that is crucial for performance of the task.

In terms of anatomical abnormalities, the caudate nucleus has been found to be consistently enlarged in autism (Stanfield *et al.*, 2008) and interestingly, the degree of caudate enlargement has been associated with the severity of restricted, repetitive behaviours (Hollander *et al.*, 2005; Rojas *et al.*, 2006). The reduced caudate activity in the ASD group in this study may be linked to structural abnormalities of this brain region.

5. Conclusion

In this study, the visuospatial process of mental rotation was studied in individuals with ASD. Behavioural results revealed a previously unreported

dissociation between ASD and control groups in response times. Controls slowed significantly on the more cognitively demanding Mirror Trial-type compared to Same Trial-type but the ASD group performed the two Trial-types at the same speed. Functional MRI results indicated that brain activation during mental rotation is abnormal in a number of brain regions. The ASD group showed a significant reduction of activation in frontal, parietal, occipital, temporal and cerebellar regions. The differences in behavioural results and functional anatomy suggest that the cognitive strategies employed by the two groups during mental rotation are different. The ASD group successfully performed the mental rotation task despite significantly reduced cortical involvement, a finding in line with previous neuroimaging research using visuospatial paradigms in ASD. Aberrant neural connectivity has been widely reported in ASD and may underpin the functional activation differences observed in this study. To investigate this further, a functional connectivity analysis was performed on this data and is discussed in the following chapter.

3. Atypical visuospatial processing in ASD: insights from functional connectivity analysis

1. Introduction

1.1. Functional connectivity research in ASD

Disrupted brain connectivity, characterised by long-distance underconnectivity and local overconnectivity, has been widely reported in autism spectrum disorders. There are a large number of studies demonstrating reduced functional connectivity between both long-range fronto-posterior connections (Castelli *et al.*, 2002; Luna *et al.*, 2002; Just *et al.*, 2004; Koshino *et al.*, 2005; Villalobos *et al.*, 2005; Cherkassky *et al.*, 2006; Kana *et al.*, 2006; Just *et al.*, 2007; Kana *et al.*, 2007; Kennedy and Courchesne, 2008; Kleinhans *et al.*, 2008; Koshino *et al.*, 2008; Mason *et al.*, 2008; Kana *et al.*, 2009; Monk *et al.*, 2009; Solomon *et al.*, 2009; Damarla *et al.*, 2010; Jones *et al.*, 2010; Weng *et al.*, 2010; Liu *et al.*, 2011) and non fronto-posterior connections in ASD (Villalobos *et al.*, 2005; Turner *et al.*, 2006; Kleinhans *et al.*, 2008; Wicker *et al.*, 2008; Mostofsky *et al.*, 2009; Agam *et al.*, 2010; Assaf *et al.*, 2010; Lombardo *et al.*, 2010; Monk *et al.*, 2010). In contrast, increased intra-regional neural connectivity has also been reported and this finding has been interpreted as evidence of local neural over-connectivity, e.g. (Belmonte and Yurgelun-Todd, 2003; Belmonte *et al.*, 2004). Reports of increased intra-regional functional connectivity (Monk *et al.*, 2009; Noonan *et al.*, 2009), increased task-related activation (Baron-Cohen *et al.*, 1999; Belmonte and Yurgelun-Todd, 2003; Schmitz *et al.*, 2006; Manjaly *et al.*, 2007), increased regional homogeneity (Paakki *et al.*, 2010; Shukla *et al.*, 2010a), and elevated short range coherence in MEG and EEG studies (Murias *et al.*, 2007; Perez Velazquez *et al.*, 2009; Barttfeld *et al.*, 2011) all support the theory of local over-connectivity in autism. It would appear that functional connectivity abnormalities in ASD are widespread, can be characterized by both increased and reduced patterns of connectivity and that they affect multiple cognitive functions. It has been argued that these neural connectivity dysfunctions may underlie the atypical visuospatial processing abilities of

people with ASD (e.g. (Bertone *et al.*, 2005; Caron *et al.*, 2006; Damarla *et al.*, 2010; Sahyoun *et al.*, 2010b)).

As discussed in the previous chapter, a large number of behavioural and neuroimaging studies have described atypical visuospatial processing in ASD (Shah and Frith, 1983; Jolliffe and Baron-Cohen, 1997; Plaisted *et al.*, 1998b; O'Riordan and Plaisted, 2001; Caron *et al.*, 2004; Pellicano *et al.*, 2005; Caron *et al.*, 2006; Silk *et al.*, 2006; Dawson *et al.*, 2007; Lee *et al.*, 2007b; Manjaly *et al.*, 2007; Joseph *et al.*, 2009; Damarla *et al.*, 2010; Soulieres *et al.*, 2011) and behavioural and functional MRI results reported in the previous chapter indicate atypical visuospatial processing in ASD during mental rotation.

To test the theory that connectivity abnormalities underpin atypical visuospatial processing, this study uses a well-characterized mental rotation task, as described in the preceding chapter (section 2.2). This task utilizes a distributed neural network, allowing for an investigation of the nature of aberrant functional connectivity during visuospatial processing in ASD.

1.2. The effect of abnormal functional connectivity on visuospatial processing in ASD

ASD is associated with abnormal neural circuitry in terms of both short and long-range connectivity. In addition to functional evidence from fMRI, EEG and MEG studies, post-mortem histological studies (Casanova *et al.*, 2006; Hutsler and Zhang, 2010) also suggest that over-connectivity may exist within local networks in ASD. Local over-connectivity might lead to the development of hyperspecialised intraregional networks and a subsequent low-level visual processing bias (Just *et al.*, 2004). In keeping with this idea, the Enhanced Perceptual Functioning (EPF) theory (Mottron *et al.*, 2006) suggests that low-level (posterior and central) visual cortical regions are involved in superior object discrimination and identification in autism, compared with typically developing controls (Grill-Spector and Malach, 2004). Similarly, the Weak Central Coherence (WCC) theory suggests that there is superior local processing in ASD, possibly combined with a deficit in global integration of information (Happe and Frith, 2006). Possible causes for this proposed visual cortical superiority include hyperspecialisation of

low-level processing areas secondary to short-range overconnectivity (Just *et al.*, 2004; Wass, 2011) or excess lateral inhibition, a process that plays a crucial role in determining object orientation, in the visual cortex (Bertone *et al.*, 2005; Vandenbroucke *et al.*, 2008). Putative pathophysiological mechanisms for excess lateral inhibition in ASD include altered structural organization of minicolumns (Casanova *et al.*, 2002; Casanova *et al.*, 2006) or an imbalance of excitatory and inhibitory neurotransmitters (Hussman, 2001; Bourgeron, 2009). In addition, reduced long-range connectivity between executive and low-level processing regions has been consistently reported in ASD (Castelli *et al.*, 2002; Luna *et al.*, 2002; Just *et al.*, 2004; Koshino *et al.*, 2005; Villalobos *et al.*, 2005; Cherkassky *et al.*, 2006; Kana *et al.*, 2006; Just *et al.*, 2007; Kana *et al.*, 2007; Kennedy and Courchesne, 2008; Kleinhans *et al.*, 2008; Koshino *et al.*, 2008; Mason *et al.*, 2008; Kana *et al.*, 2009; Monk *et al.*, 2009; Solomon *et al.*, 2009; Damarla *et al.*, 2010; Jones *et al.*, 2010; Weng *et al.*, 2010; Liu *et al.*, 2011). This long distance underconnectivity may impact on visuospatial functioning in ASD by disrupting top-down higher-level processing. Top-down processing in ASD may also be disrupted by dysfunction of executive control regions, a phenomenon that is well recognized in ASD (Russell *et al.*, 1991; Hughes and Russell, 1993; Hughes *et al.*, 1994; Ozonoff *et al.*, 1994; Townsend *et al.*, 1996a; Townsend *et al.*, 1996b; Ozonoff and Jensen, 1999; Townsend *et al.*, 1999; Verte *et al.*, 2005; Rinehart *et al.*, 2006; Steele *et al.*, 2007). It has been suggested that a reduction in top-down influences on visuospatial processing in ASD may result in less interference with low-level perceptual processing and could therefore confer a relative visuo-spatial processing advantage to individuals with ASD (Liu *et al.*, 2011).

1.3. Psychophysiological interactions (PPI) analysis

Given the extensive evidence for disordered connectivity in ASD, the aim of this study was to investigate levels of network synchronisation during mental rotation in order to increase understanding of the pathophysiology of both atypical visuospatial processing and aberrant interregional connectivity in ASD. A psychophysiological interactions (PPI) analysis was used to interrogate functional connectivity during mental rotation.

1.3.1. Introduction to PPI

Psychophysiological interactions (PPI) analysis is a method in brain imaging for investigating task-dependant functional connectivity (Friston *et al.*, 1997). In a typical functional connectivity analysis, the level of synchronization between brain regions is measured by looking at statistical associations between BOLD intensity time-series for different brain regions during the entire psychological task. Such a connectivity analysis essentially calculates how "in synch" two regions are, in the time series. A PPI analysis is a type of functional connectivity analysis that allows for the investigation of how brain regions interact specifically during a psychological task (or during selected conditions of the task) by measuring task-dependant interregional co-variance. In other words, PPI measures the functional connectivity between a brain region and the rest of the brain in relation to the performance of a psychological task. This method therefore allows for an assessment of how the activity within brain networks is modulated by varying psychological states within an fMRI task.

It was felt that a PPI analysis would be particularly suitable in this study as a specific aim was to examine how ASD participants, relative to control participants, differed in functional connectivity specifically during the Same and Mirror Trial-types of the mental rotation task. This analysis was planned in order to investigate the neural correlates of the striking dissociation between groups in response times on Same and Mirror Trial-types that was reported in the previous chapter (section 3.1.1). In summary, the Control group slowed significantly during the Mirror Trial-type compared with the Same Trial-type whereas the ASD group showed no slowing on the Mirror Trial-type. This finding suggested that visuospatial processing mechanisms in the ASD group were atypical. The aim of this PPI analysis was to examine between-group differences in functional connectivity on each of these (Same / Mirror) Trial-types to increase our understanding of the underlying pathophysiology of atypical visuospatial processing in ASD.

1.3.2. Fundamentals of PPI analysis

Activity in PPI analysis is modeled using three explanatory regressors, including a voxel time-course as the physiological brain response variable; a

psychological variable based on the context of the stimuli presented; and the psychophysiological product of the former two variables (the psychophysiological interaction regressor). The first two regressors are treated as regressors of no interest and the third is used to generate statistical maps.

If two brain regions are interacting, then the level of activity in those areas will correlate over time. If the voxel time-series is extracted from a seed region of interest (regressor 1 above) and used as an explanatory variable in a General Linear Model (GLM), brain regions with a high z-score are the ones whose time-series correlate most closely, i.e. those that vary their activity most "in synch" with the seed region. Interactions between brain regions may vary however, according to different psychological contexts/tasks, which would result in a change in correlation between the time-series in those areas. PPI analysis looks for brain regions that have a higher correlation with the seed region time-series specifically during a particular psychological task, by adding in regressor 2, the task time-course to the model. There are a number of reasons for why time-series from different brain regions may correlate during a psychological task. For example, regions that are anatomically connected or that share neuromodulatory or sensory inputs will all have time-series that correlate. A simple analysis of functional connectivity will identify brain regions whose time-series correlate most closely with that of the seed region during certain time periods and this may reveal brain regions that are "interacting", even if the correlation has nothing to do with the psychological task in the experiment.To overcome this difficulty, the psychophysiological interaction (PPI) regressor is created and this regressor is entered as the explanatory variable into the GLM. As mentioned above, the PPI regressor is generated by multiplying the task time-course (psychological component) by the time-series from the seed region (physiological component) (see Figure 1). The interaction regressor is generated as the product of the physiological and psychological 'main effects' variables; consequently these variables, the time-series from the seed region and from the task, are also included in the GLM as covariates of no interest. This is to ensure that the variance

associated with the interaction regressor exceeds that accounted for by the main effects of physiological and psychological correlation. Voxels with a high z-score from this GLM analysis are those with a time-series that show more correlation with the seed region time-series during the task of interest than at other times. In other words, this analysis can identify a region, A, of the brain that is active in response to a stimulus only when another region, B, of the brain is active.

1.4. Aims and Hypothesis

The aim of this PPI study was to investigate between-group differences in functional connectivity during Same and Mirror Trial-types of a mental rotation task in order to examine the neural correlates of atypical visuospatial processing in ASD. Behavioural results reported in the previous chapter revealed a striking dissociation between groups in response times. The Control group slowed significantly on Mirror trials relative to Same trials whereas the ASD group showed consistent response times on the two Trial-types. This may reflect a relative superiority of processing of Mirror Trial-type in the ASD group, a finding in keeping with the Enhanced Perceptual Functioning (EPF) theory of autism (Mottron *et al.*, 2006). Although this EPF theory does not contain a specific neurobiological explanation of superior perceptual functioning in autism, two possible causes are noted. First, a reduction in interference from higher level, top-down processing may occur, due to disrupted long range neural connections. Second, hyper-specialisation of early perceptual regions may occur, due to local overconnectivity or excess lateral inhibition. Based on the large body of literature reporting abnormal functional connectivity in ASD, it was hypothesized that functional connectivity would be abnormal during mental rotation. Specifically, we hypothesized that long range fronto-posterior connections would be compromised in the ASD group, and that short-range intraregional connectivity in posterior regions would be increased in individuals with ASD.

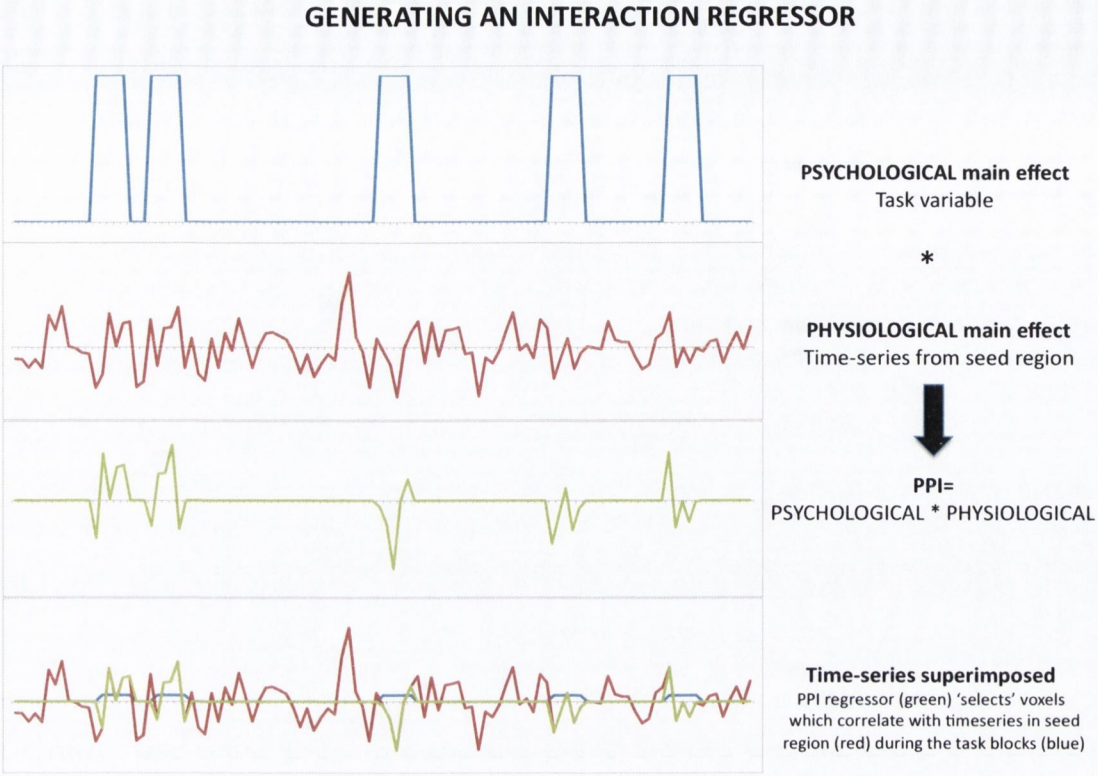


Figure 1: Generation of the PPI interaction regressor. To create the interaction regressor, the task timeseries is multiplied by the de-meaned physiological timeseries. The task timeseries is coded with "+1"s for all repetition times (TRs) during the task of interest, and either "-1"s or "0"s at all other times. When the aim of the PPI analysis is to analyze differences in connectivity between two psychological task types within-subjects, a +1/-1 coding is required. When comparing connectivity during one task condition between groups, a +1/0 coding is appropriate. (Figure adapted from http://www.fmrib.ox.ac.uk/Members/joreilly/what-is-ppi)

2. Methods

Functional MRI data were collected during performance of a mental rotation task. Details of participants, the mental rotation paradigm, functional MRI image acquisition, behavioural analysis and functional MRI analysis are all outlined in the methods section of the preceding chapter (sections 2.1 – 2.4).

2.1. PPI analysis

PPI connectivity analysis (Friston *et al.*, 1997) was used to examine the functional connectivity between seed regions of interest and the rest of the brain during task performance. Six seed regions of interest (ROIs) were selected: inferior frontal (right BA8), temporal (right fusiform), occipital (right cuneus and left BA19), cerebellar (left inferior semilunar lobule) and striatal (right caudate). These seed ROIs were the largest clusters showing between-group activation differences on fMRI analysis in five major regions of the brain; frontal, temporal, occipital, cerebellar, striatal (see chapter 2, section 3.2.2) and are regions known normally to be required for mental rotation (see Figure 2). For each seed ROI, an 8mm radial sphere was generated, centered at the centre of mass of the cluster showing a between-group difference in activation on fMRI analysis. PPI analyses were performed separately for each ROI for the Same and Mirror trials for each Group.

For each PPI analysis, the multiple regression analysis (for fMRI analysis) described in the previous chapter (section 2.4.2) was rerun for each subject, but with the insertion of two additional regressors. The first regressor, the physiological variable, was the de-trended subject-specific time-course of activity in the ROI (averaged across all voxels in the 8mm sphere). The second regressor - the psychophysiological interaction term - was created by calculating the product of the de-trended activation time-course from the seed region and the task regressor (which contained "1"s for all repetition times (TRs) during which the Trial-type of interest occurred, and "0"s for all other TRs). The parameter estimate for the interaction term was converted to a z-score through Fisher transformation for each subject.

To investigate between-group differences in functional connectivity, z-scores from each PPI analysis were entered into a two-way repeated measures ANOVA [Group (ASD/Control) x Trial-type (Same/Mirror)]. For this analysis voxels were required to pass a voxelwise statistical threshold set at $P \leq 0.001$ and to be part of a larger 162 μL cluster of contiguous significant voxels. This cluster size was determined through a Monte Carlo simulation and resulted in less than a 1% probability of significant activation within a cluster surviving due to chance. This more conservative voxelwise threshold was used as extensive activations were observed at the $P \leq 0.005$ threshold. For each of the ROIs showing a significant Group x Trial-type interaction effect, the average activation value for each cluster was extracted, imported into SPSS and subjected to pairwise comparisons.

The dependant variables that result from this PPI analysis are negative and positive connectivity. Negative connectivity indicates that the influence the task has on activity in the seed region produces a correlated opposite effect on the projection region, which is consistent with (but not proof of) one region suppressing the other. In contrast positive connectivity between a pair of brain regions indicates that as activity in one brain region increases, there is a correlated increase in activity in the other region.

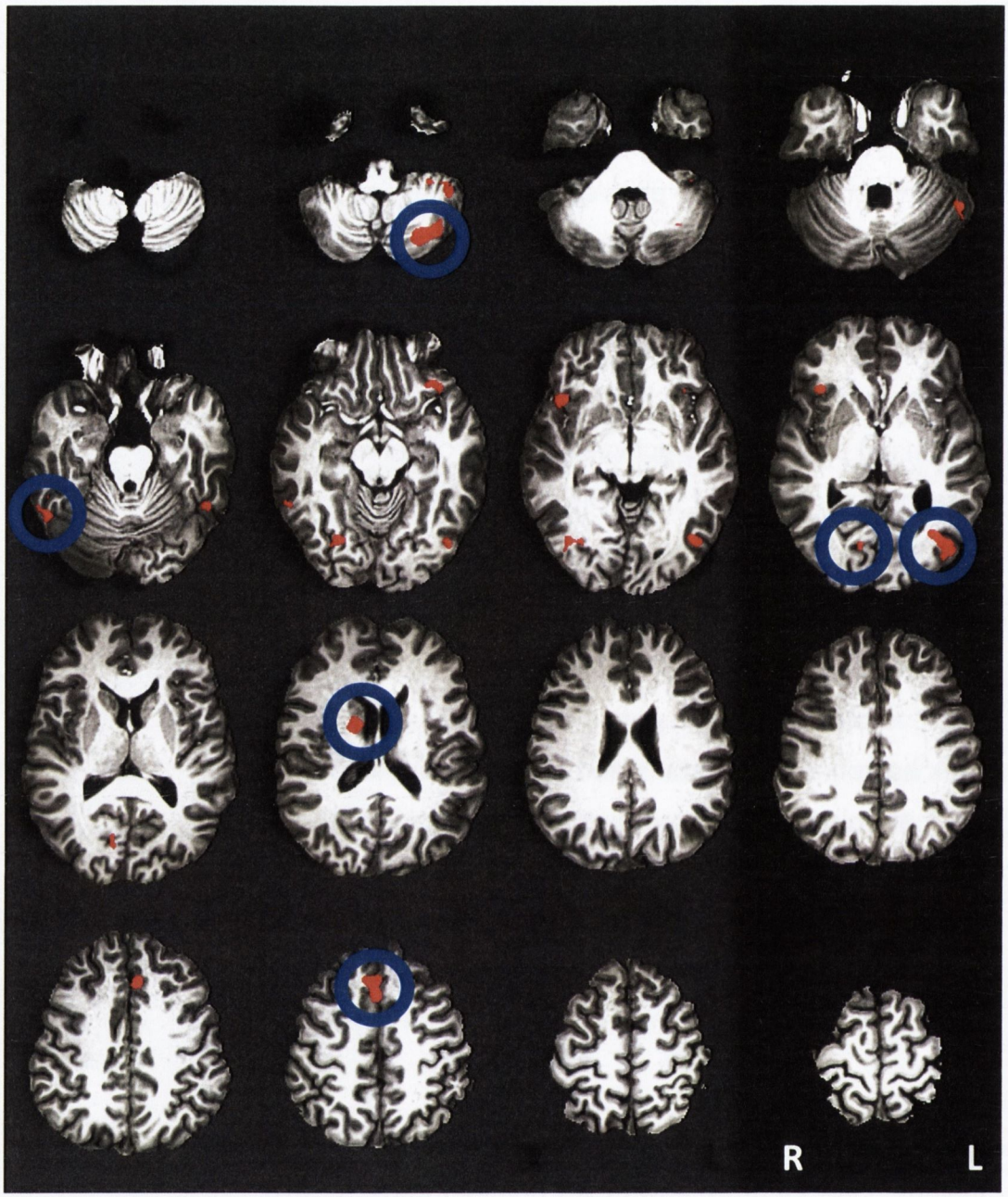


Figure 2: Axial sections showing regions of significant activation differences (red) between Control and ASD groups during mental rotation ($p<0.05$, corrected). All regions showed greater activation in the Control group. Blue circles indicate the 6 seed regions selected for functional connectivity analysis; from top-left: left inferior semilunar lobule, right fusiform gyrus, right cuneus, left BA19, right caudate, right BA8.

3. Results

PPI analysis was carried out for six seed ROIs in both the Same and Mirror Trial-types and a two way voxel-wise ANOVA was performed for each ROI [Group (ASD/Control) x Trial-type (Same/Mirror)].

3.1 Main effect of Group

There was a significant main effect of Group for all six PPI analyses (Table 1). Overall, two patterns of between-group difference were evident. Compared with controls, the ASD group showed either significantly reduced functional connectivity [either positive or negative connectivity (pattern 1)] or the opposite pattern of functional connectivity [e.g. negative connectivity in controls and positive connectivity in the ASD group (pattern 2)].

Relative to the ASD group, controls showed stronger negative functional connectivity (pattern 1) between right fusiform / left BA19 / left inferior semilunar lobule seed regions and multiple interhemispheric and inter-regional areas. Controls also showed stronger positive connectivity than the ASD group (pattern 1) between right cuneus/right caudate seed regions and a number of brain regions (see Table 1 and Figure 3). Pattern 2 was observed predominantly for seed regions in right BA8 and right cuneus. Controls showed positive connectivity between the right cuneus and parietal/temporal regions whereas the ASD group showed negative connectivity between these regions. Conversely, controls showed negative connectivity between right BA8 and frontal, parietal, temporal, occipital, cerebellar and striatal regions whereas the ASD group showed positive connectivity between these regions (see Table 1 and Figure 3).

Seed ROI			Cluster location	BA	Voxels	X	Y	Z	Effect
Right BA8	Frontal	L	Medial Frontal Gyrus	6	2024	1.6	-4.5	52.3	C - ASD +
		L	Inferior Frontal Gyrus	47	1154	22	-30.4	2.2	C - ASD +
		L	Superior Frontal Gyrus	11	253	23.6	-44	-18.2	C - > ASD -
		L	Inferior Frontal Gyrus	47	187	50.2	-25.8	-7	C + > ASD+
		R	Superior Frontal Gyrus	11	162	-27	-42.6	-16.7	C - ASD +
	Parietal	L	Precuneus	7	673	27.4	55.7	51.5	C - ASD +
	Cingulate	L	Posterior Cingulate	23	331	1.1	32	23.1	C - ASD +
	Temporal	L	Superior Temporal Gyrus	38	461	34.9	-15.9	-21.9	C - ASD +
		L	Fusiform Gyrus		277	28.9	58.1	-9.5	C - ASD +
	Occipital	L	Cuneus	19	1330	18.7	85.3	30.1	C - ASD +
			Cuneus	17	222	1.4	91.8	2.8	C - ASD +
	Cerebellum	L	Culmen		244	10.1	48.2	-0.1	C - ASD +
	Subcortical	L	Thalamus		1106	1.4	19.8	9.6	C - ASD +
Right Fusiform	Frontal	R	Precentral Gyrus	4	170	-32	21.5	60.6	C - > ASD -
	Parietal	L	Postcentral Gyrus	1	346	33.8	33.9	66.4	C - ASD +
	Temporal	L	Parahippocampal Gyrus	20	162	43.2	14.7	-15.7	C - > ASD -
	Subcortical	L	Pons		264	8.9	16.7	-35.2	C - ASD +
Right Cuneus	Parietal	L	Inferior Parietal Lobule	40	404	55.5	53.9	39	C + > ASD +
		R	Inferior Parietal Lobule	40	222	-58.1	32.2	28.2	C + ASD -
		R	Supramarginal Gyrus	39	180	-53.8	58	30.1	C + > ASD +
	Temporal	L	Superior Temporal Gyrus	39	1439	51.2	61.2	20.5	C + ASD -
		R	Middle Temporal Gyrus	22	720	-52	39.1	5.4	C + ASD -
Left BA19	Frontal	R	Middle Frontal Gyrus	6	977	-18.3	10	59.1	C - > ASD -
		R	Medial Frontal Gyrus	6	935	-1.8	-3.6	51.5	C - > ASD -
	Parietal	L	Postcentral Gyrus	3	541	46.2	15.4	52.2	C - > ASD -
	Temporal	L	Uncus	20	165	28.3	-0.1	-33.1	C - ASD +
	Occipital	L	Cuneus	18	657	19.2	84.4	26.1	C - > ASD -
		R	Cuneus	17	459	-4.9	91.5	4.7	C - ASD +
		R	Cuneus	18	177	-16.1	86.8	19.2	C - ASD +
	Subcortical	L	Caudate		1198	10.2	-7	4.7	C - > ASD -
		L	Thalamus		1026	4.6	23.1	8.9	C - > ASD -
		L	Caudate		337	24.5	18.3	28.9	C - ASD +
		R	Caudate		598	-13.4	-13.6	12.1	C - > ASD -
		R	Lentiform		233	-17.6	-4.2	-4.6	C - > ASD -
Left Inferior Semilunar Lobule	Frontal	L	Precentral Gyrus	6	347	48.4	-2.3	34.4	C - > ASD -
		R	Middle Frontal Gyrus	6	653	-27.5	7.4	48.5	C - > ASD -
	Parietal	L	Precuneus	7	255	17.2	77.1	45.7	C - > ASD -
		L	Superior Parietal Lobule	7	173	25.3	59.9	55.7	C - > ASD -
		R	Superior Parietal Lobule	7	325	-22.9	65.1	46.4	C - > ASD -
	Occipital	L	Cuneus	19	566	21.3	88.2	25.4	C - > ASD -
Right Caudate	Temporal	R	Superior Temporal Gyrus	22	317	-62.2	44.4	6.2	C + ASD -
	Cerebellar	L	Cerebellar Tonsil		380	34.9	35.6	-44.1	C + > ASD +
		L	Cerebellum (XIII)		347	41.8	50.6	-51.9	C + > ASD +
	Subcortical	L	Thalamus		218	6.1	18.7	8	C - > ASD -

Table 1: Location and size of clusters showing a significant main effect of Group in PPI analysis for each seed region of interest. (Direction of between-group difference denoted with arrows (- = Negative connectivity, + = Positive connectivity), > = greater than (note that when both groups show negative connectivity the > means a larger negative value), R = Right, L = Left, BA = Brodmann Area, C = Control group, ASD = Autism Spectrum Disorder group. Shading is for visualization to separate the seed regions).

3.2. Main effect of Trial-type

There was a significant main effect of Trial-type in 4 of the 6 PPI analyses (right BA8, right cuneus, left BA19 and right caudate). Three of these analyses (right BA8, right cuneus and right caudate seed regions) showed a very consistent pattern characterized by greater functional connectivity on Mirror trials than Same trials. There were multiple projection regions in frontal, parietal, temporal, occipital, cerebellar and striatal regions. The main effect of Trial-type in the PPI analysis of left BA19 was more varied with most clusters (in multiple frontal, parietal, occipital, cerebellar and striatal regions) showing greater connectivity on Mirror trials, but some (e.g. left insula, bilateral inferior parietal lobule) showing greater connectivity on Same trials. These results are outlined in detail in Table 2.

Seed ROI			Cluster location	BA	Voxels	X	Y	Z	Group difference
Right BA8	Parietal	R	Precuneus	19	267	-36.9	72.3	38.5	M + > S +
		R	Inferior Parietal Lobule	40	233	-44.4	54.1	40.3	M + > S +
	Cingulate	R	Cingulate Gyrus	31	246	-20.1	45.5	23.7	M + > S +
	Temporal	R	Superior Temporal Gyrus	39	283	-42.6	54.9	25.8	M + > S +
		R	Insula	13	195	-29.4	-14.3	10.8	M - > S -
	Occipital	L	Middle Occipital	19	250	28.9	65.1	7.3	M + > S +
	Cerebellar	L	Uvula		537	0.4	75.6	-34.1	M + > S +
	Subcortical	L	Caudate		371	11.8	-7.4	11.7	M - > S -
Right Cuneus	Frontal	L	Subcallosal Gyrus	34	185	22.7	-5.3	-10	M - > S -
	Occipital	R	Cuneus	17	2160	-21.5	93.8	3.7	M + > S +
		L	Inferior Occipital Gyrus	18	180	40.2	84.6	-16.1	M + > S +
	Cerebellum	R	Declive/Fusiform		3344	-31.8	72	-16.3	M + > S +
		L	Tuber		563	17.4	85.6	-29.4	M + > S +
		L	Inferior Semilunar Lobule		489	4.3	66	-51.9	M + > S -
		R	Cerebellum (crus 2)		328	-28.8	74.3	-51.5	M + > S +
		L	Inferior Semilunar Lobule		182	35	67.4	-48.7	M + > S +
Left BA19	Frontal	L	Medial Frontal Gyrus	10	525	0.5	-56.3	4.3	M - > S -
		L	Superior Frontal Gyrus	10	338	18.7	-53.7	6.8	M - > S +
		L	Insula	13	320	40.5	4.3	16.6	S - > M -
		R	Middle Frontal Gyrus	9	389	-36.3	-41.8	37.7	M + > S -
		R	Middle Frontal Gyrus	9	389	-36.3	-41.8	37.7	M + > S -
		R	Anterior Cingulate	32	201	-14	-39.2	9.4	M - > S -
		R	Middle Frontal Gyrus	9	190	-48.1	-35.7	30.6	M + > S -
	Parietal	L	Inferior Parietal Lobule	40	457	47	46.5	52.3	S - > M -
		L	Inferior Parietal Lobule	40	338	56	36.7	44.8	S - > M -
		R	Superior Parietal Lobule	7	291	-23.9	65	44.4	S - > M -
		R	Inferior Parietal Lobule	40	288	-36.6	54.4	42.7	M + > S -
	Occipital	R	Middle Occipital Gyrus	18	1638	-29	77.2	3.5	M + > S +
	Cerebellum	L	Pyramis		352	0.7	75.1	-30.3	M + > S +
		R	Culmen		1273	-29.8	53.9	-23.9	M + > S -
		R	Pyramis		372	-44	64.8	-31.5	M + > S +
		R	Pyramis		239	-23.7	72.8	-26.6	M + > S +
	Subcortical	L	Lentiform Nucleus		955	22.5	-11.1	2.2	M - > S -
		R	Lentiform Nucleus		274	-23.8	-10.9	1.1	M - > S -
Right Caudate	Parietal	R	Inferior Parietal Lobule	7	1124	-31.5	48.5	40.4	M + > S +
	Occipital	L	Cuneus	17	972	23.4	91.4	-0.5	M + > S +
		L	Lingual Gyrus	18	190	28.4	71.8	-9.3	M + > S -
		R	Inferior Occipital Gyrus	18	226	-41	86.5	-6	M + > S +
		R	Middle Occipital Gyrus	19	193	-32.6	88.5	9.9	M + > S +
	Cerebellar	L	Inferior Semilunar Lobule		1401	-0.3	70.8	-38.3	M + > S +
		L	Uvula		608	18.4	82.1	-23.4	M + > S +
		L	Declive		421	35	70.3	-21.1	M + > S +
		L	Pyramis		192	43.3	65.9	-31.8	M + > S +
	Subcortical	L	Lentiform Nucleus		241	18.2	-12.3	6.7	M - > S -
		R	Lentiform Nucleus		273	-21.4	-11.5	0.6	M - > S -

Table 2: Location and size of clusters showing a significant main effect of Trial type in PPI analysis for each seed region of interest. Direction of between-group difference denoted with arrows (R = Right, L = Left, BA = Brodmann Area, - = Negative connectivity, + = Positive connectivity, M = Mirror Trial type, S = Same Trial type).

3.3. Group x Trial-type interaction

For all six PPI analyses, there was a significant Group x Trial-type interaction (see Table 3 and Figure 3). To investigate what was driving the interaction, pairwise comparisons were performed in SPSS on the ROI-level for each cluster. A one-sample t-test against zero was also performed to confirm that connectivity levels were significantly greater than zero. As task difficulty increased (i.e. from Same to Mirror trials), the interaction effects were, in general, characterized by an increase in negative connectivity in the ASD group and either a reduction in negative connectivity or an increase in positive connectivity in controls. The specific results from each seed region are discussed below (see Table 3 and Figure 3). The pattern of dissociation between groups that is revealed by this Group x Trial-type interaction is similar to that reported in the behavioural results and these between-group differences in connectivity may represent the neural underpinnings of the behavioural results.

3.3.1. Right BA8

Voxelwise analysis showed a significant Group x Trial-type interaction for one cluster in the left inferior frontal gyrus. Pairwise comparisons of the Mirror trials relative to Same trials revealed that controls showed an increase in positive connectivity whereas the ASD group showed an increase in negative connectivity. Between-group pairwise comparisons revealed a significant between-group difference on Mirror trials with positive connectivity in controls and negative connectivity in the ASD group.

3.3.2. Right Cuneus

Voxelwise analysis revealed a significant Group x Trial-type interaction with 5 clusters in the right precuneus (x2), left insula, right BA19 and right cuneus. On Mirror trials compared with Same trials, the ASD group showed a significant increase in negative connectivity (right precuneus x 2, left insula) and positive connectivity (right cuneus). Comparatively, controls showed a significant reduction in negative connectivity (right precuneus) and positive connectivity (right cuneus). Pairwise comparisons also showed significant between-group differences on Same trials in one cluster (right precuneus - negative connectivity in controls, no significant connectivity in

the ASD group) and on Mirror trials in three clusters; relative to controls, the ASD group showed greater negative connectivity (right precuneus, left insula) or greater positive connectivity (right cuneus).

3.3.3. Left BA19

There was a significant Group x Trial-type interaction for clusters in the right middle frontal gyrus and left inferior frontal gyrus. On Mirror trials, the ASD group showed an increase in negative connectivity (left inferior frontal gyrus) or reduction in positive connectivity (right middle frontal gyrus) whereas the control group showed an increase in positive connectivity (left inferior frontal gyrus) or reduction in negative connectivity (right middle frontal gyrus). There was a significant between-group difference in one cluster on Same trials (right middle frontal gyrus - positive connectivity in the ASD group but negative connectivity in controls) and in one cluster on Mirror trials (left inferior frontal gyrus - positive connectivity in controls but negative connectivity in the ASD group).

3.3.4. Right Fusiform

There was a significant Group x Trial-type interaction for six clusters (right inferior frontal gyrus, left middle temporal gyrus, right uvula, left declive x 2, left culmen). In all clusters, on Mirror trials relative to Same trials, the ASD group showed an increase in negative connectivity whereas controls showed a reduction in negative connectivity. There were significant between-group differences on Same trials (stronger negative connectivity in controls in all clusters except the right uvula) and Mirror trials (greater negative connectivity in the ASD group in two clusters - left culmen, right uvula).

3.3.5. Left Inferior Semilunar Lobule

There was a significant Group x Trial-type interaction for one cluster in the left lingual gyrus. On Mirror trials compared with Same trials, the ASD group showed an increase in negative connectivity whereas the control group showed a reduction in negative connectivity. There was a significant between-group difference in connectivity on Same trials with negative connectivity in controls but no significant connectivity in the ASD group.

3.3.6. Right Caudate

There was a significant Group x Trial-type interaction for two clusters (right middle temporal gyrus, right anterior cingulate). On Mirror trials the ASD group showed reduced positive connectivity between right caudate and right anterior cingulate and switched from positive connectivity to negative connectivity between right caudate and right middle temporal gyrus. In contrast, for controls the levels of connectivity between the right caudate and both clusters were not significantly different from zero, during both Same and Mirror trials. There was a significant between-group difference on Same Trial-type in both clusters (positive connectivity in the ASD group but no significant connectivity in controls) and on Mirror Trial-type in one cluster (right middle temporal gyrus) with negative connectivity in the ASD group but no significant connectivity in controls.

Seed ROI		Location	BA	Voxels	X	Y	Z	ASD (M v S)	C (M v S)	Same (ASD v C)	Mirror (ASD v C)
FRONTAL											
Right BA8	L	Inferior Frontal gyrus	47	231	34.7	-21.6	-9.7	↑ - **	↑ + **		C + ASD - ***
OCCIPITAL											
Right Cuneus	R	Precuneus	7	227	-8.6	67.5	44.2	↑ - *	↓ - **	C - ASD 0 *	
	R	Precuneus	7	218	-23.4	68.8	36.5	↑ - *			C 0 ASD - *
	R	Middle Occipital gyrus	19	421	-38	80	12.7				
	R	Cuneus	19	162	-1.5	90.8	31.5	↑ + ***	↓ + **		C 0 ASD - **
	L	Insula	13	181	40	-7.2	14.9	↑ - **	S 0 M 0 **		C+ < ASD + ***
Left BA19	R	Middle Frontal gyrus	46	182	-47.1	-38.6	20.7	↓ + **	↓ - **	C - ASD + **	
	L	Inferior Frontal gyrus	47	192	35.1	-19.6	-8.9	↑ - **	↑ + **		C + ASD - **
TEMPORAL											
Right Fusiform	R	Inferior Frontal gyrus	9	214	-47.6	-5.6	23.9	↑ - **	↓ - **	C - > ASD - **	
	L	Middle Temporal gyrus	21	195	61.1	51.3	7.9	↑ - **	↓ - **	C - ASD 0 **	
	R	Uvula		320	-23.2	68	-24.7	↑ - *	↓ - **		C 0 ASD - *
	L	Declive		449	38.2	59.4	-15.6	↑ - **	↓ - **	C - ASD 0 **	
	L	Culmen		402	20	59.8	-25.8	↑ - **	↓ - ***	C - ASD 0 **	C 0 ASD - *
	L	Declive		281	9.5	71.7	-15.2	↑ - *	↓ - **	C - ASD 0 **	
CEREBELLAR											
Left Inferior Semilunar Lobule	L	Lingual gyrus		246	60.9	47.5	14.5	↑ - **	↓ - ***	C - ASD 0 **	
SUBCORTICAL											
Right Caudate	R	Middle Temporal gyrus	21	247	-56.7	7.6	-18.7	↑ - ***		C 0 ASD + **	C 0 ASD - *
	R	Anterior Cingulate	11	292	1.3	-32.7	-5.9	↓ + **		C 0 ASD + **	

Table 3: Caption on following page

Table 3 caption: Group x Trial-type interaction results and pairwise analysis summary for PPI analyses from 6 seed regions (right BA8, right cuneus, left BA19, left inferior semilunar lobule, right fusiform and right caudate). To illustrate how the groups differ in connectivity on Mirror trials relative to Same trials, the direction of change in connectivity on Mirror trials relative to Same trials is presented for each group in the ASD (M v S) and C (M v S) columns. To illustrate between group differences in connectivity, the direction of connectivity in control and ASD groups is presented for each Trial-type in the Same (ASD v C) and Mirror (ASD v C) columns. (M=Mirror Trial-type; S=Same Trial-type, C=Control group, ASD=ASD group, ↑ = increased, ↓= reduced, - = negative connectivity, + = positive connectivity, 0 = no significant level of connectivity. *Indicates significance level of pairwise comparisons (* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$)

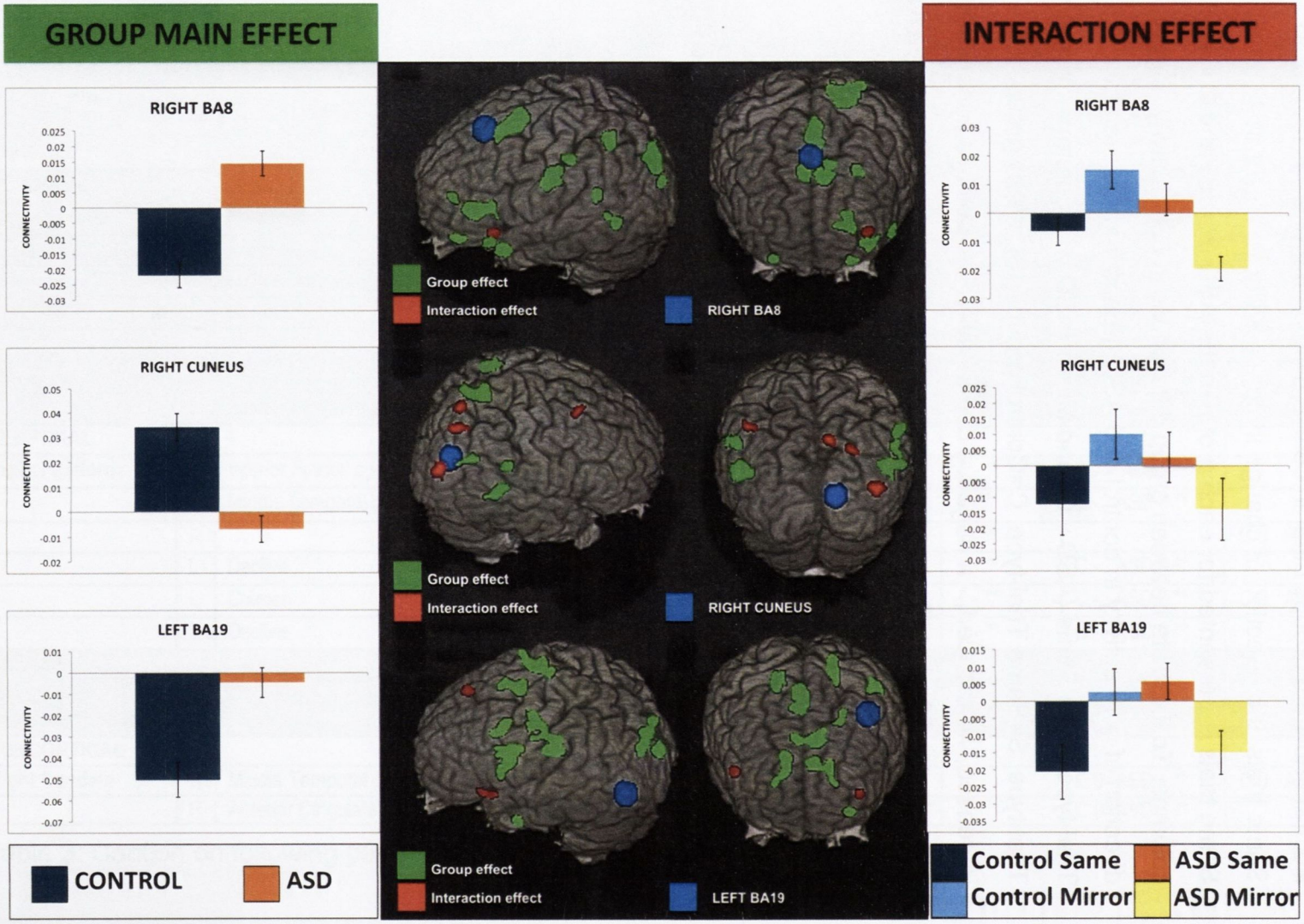


Figure 3a: Caption after Figure 3b

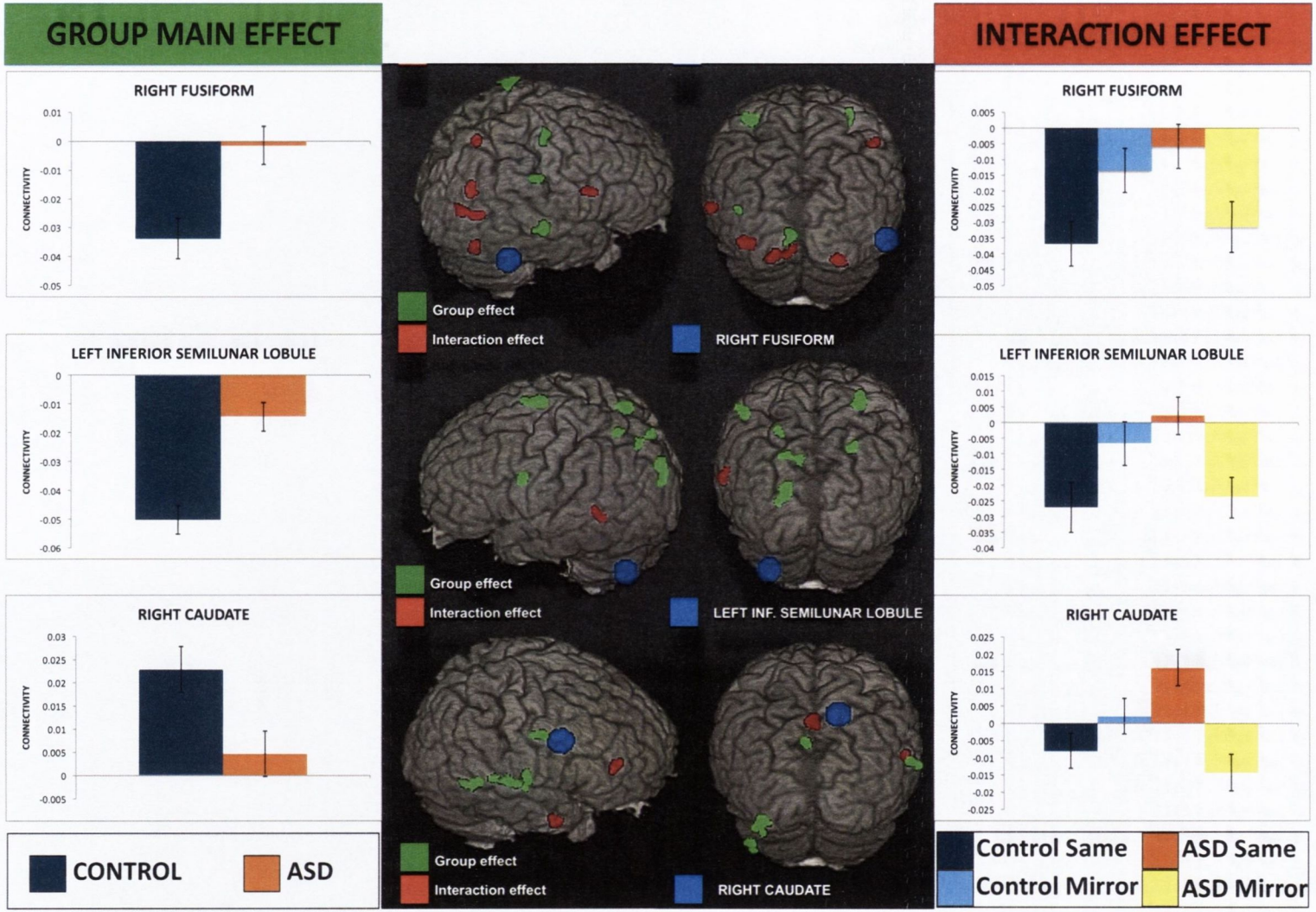


Figure 3b: Caption on following page

Figure 3a and 3b caption: Group main effect and Group x Trial-type interaction results for PPI analysis from three seed regions (right BA8, right cuneus, left inferior semilunar lobule, right fusiform, left BA19 and right caudate) showing significant between-group differences in functional connectivity (green regions – Group main effect; red regions – interaction effect) for each seed region (blue). Bar graphs illustrate the average strength of connectivity for each Group (Control/ASD) in the significant clusters in the Group main effect analysis and for each Group (Control/ASD) and Trial-type (Same / Mirror) in the significant clusters in the Group x Trial-type interaction analysis. For ease of exposition, only the average strength of connectivity between the seed ROI and significant clusters is shown in the bar graphs. Connectivity measures were also analysed region by region and the same pattern of effects was observed when each connectivity region was graphed separately (see Table 3).

4. Discussion

This PPI study aimed to investigate the neurocognitive basis of mental rotation differences between individuals with ASD and typically developing controls from the perspective of functional connectivity.

As discussed in the previous chapter, behavioural analysis revealed a striking and previously unreported dissociation between the ASD and Control groups in response times for Same and Mirror Trial-types. Controls took significantly longer on the Mirror Trial-type than on the Same Trial-type, whereas the ASD group performed the two Trial-types at a similar speed. Functional MRI results (also discussed in the previous chapter) revealed that in comparison with the Control group, the ASD group had significantly reduced brain activity in multiple brain regions known to be involved in mental rotation including frontal (BA 47, BA 8), temporal (BA 37), occipital (BA 18, 19), striatal and cerebellar regions. Previous fMRI studies have reported similar findings of successful visuospatial task performance despite an overall reduction in cortical activation (Silk *et al.*, 2006; Lee *et al.*, 2007b). In these studies, no specific explanation was offered for how successful task performance could be achieved with such reduced cortical involvement, but the results would suggest that visuospatial processing in ASD is accomplished in an atypical way. In addition, both groups showed significantly greater activity during Mirror trials relative to Same trials. This may reflect the additional rotation required during these trials and is consistent with the theory that Mirror trials present a more demanding mental rotation task (Hamm *et al.*, 2004).

As demonstrated by the group main effect results, the PPI analyses showed significant between-group differences in functional connectivity during mental rotation. Interestingly, relative to the ASD group, controls showed stronger negative functional connectivity between the seed regions in the right fusiform, left BA19 and left inferior semilunar lobule, and multiple interhemispheric and inter-regional areas. As mentioned in the methods section, negative connectivity indicates that the influence the task has on activity in the seed region produces a correlated opposite effect on the projection region, which is consistent with (but not proof of) one region

suppressing the other. These findings may therefore suggest that controls employed greater suppression between certain regions than the ASD group during mental rotation. Most of the seed-projection pairings showing stronger negative connectivity in controls were long-range, which may suggest an inter-lobe suppression. Controls also showed stronger positive connectivity than the ASD group (pattern 1) between the right cuneus and right caudate seed regions and a number of brain regions. Interestingly, these projection regions tended to be more short-range (e.g. right cuneus with parietal regions, right caudate with temporal and thalamic regions). Overall, this widespread reduction of functional connectivity in the ASD group is consistent with a large body of literature that suggests that core deficits in ASD are associated with underconnectivity. In particular, the reduced fronto-posterior connectivity reported in the ASD group in this study (between right fusiform and right precentral gyrus, left BA19 and right middle and medial frontal gyrus, and left inferior semilunar lobule and left precentral gyrus/right middle frontal gyrus) is in keeping with the underconnectivity hypothesis of autism (Just *et al.*, 2004) and indicates a reduction of typical higher-level cortical control during mental rotation. Reduced fronto-posterior connectivity could result from abnormalities of frontal executive regions or could be a consequence of aberrant structural connectivity, for example reduced integrity of white matter tracts. Despite this reduction in functional connectivity between multiple brain regions, however, mental rotation performance was not impaired in the ASD group. One possible explanation for this is that, as a developmental consequence of impaired frontal regions or fronto-posterior connections, there may be hyperspecialisation of early perceptual regions in individuals with ASD (Mottron *et al.*, 2006; Just *et al.*, 2004).

A second pattern of between-group difference in connectivity also emerged from the Group main effect of the PPI analyses, whereby the ASD and control groups showed opposite connectivity patterns (e.g. negative connectivity in controls and positive connectivity in the ASD group or vice versa). This pattern was most evident in the PPI analysis of right BA8 seed region, although it occurred in seed-projection pairs in all other analyses,

with the exception of the cerebellar seed. Controls showed negative connectivity between right BA8 and frontal, parietal, temporal, occipital, cerebellar and striatal regions whereas in direct contrast, the ASD group showed positive connectivity between these regions. Thus while controls were suppressing these connections, the ASD group were increasing the level of connectivity between regions. This may indicate that the two groups are using qualitatively different mechanisms for mental rotation performance.

Connectivity analyses also revealed an intriguing dissociation between the ASD and control groups, as demonstrated by the interaction between Group and Trial-type. A number of seed-projection regions showed a significant interaction effect. In general, as task difficulty increased on Mirror trials, the ASD group showed an increase in negative connectivity whereas controls showed a reduction in negative connectivity or an increase in positive connectivity. This pattern of dissociation is interesting as it may illuminate the neural underpinnings of the behavioural dissociation in response times on Same and Mirror trials. The relative slowing of controls on Mirror trials was associated with a reduction in negative connectivity or an increase in positive connectivity. In contrast, the ASD group had similar response times on Same and Mirror trials, and this performance was associated with an increase in negative connectivity during Mirror trials. For example, on Mirror trials compared to Same trials, the ASD group showed stronger suppression of connections (greater negative connectivity) than controls between right BA8 and the left inferior frontal gyrus and between the left dorsal extrastriate region (BA19) and bilateral frontal regions whereas controls increased the level of positive connectivity between these pairs of regions. Similarly, the ASD group increased negative connectivity between the right fusiform and frontal regions on Mirror trials, but controls reduced the level of negative connectivity between these regions. Taken together, this suppression of frontal connectivity in ASD may represent an attempt to reduce "interference" from executive higher-level regions whose input is normally required for successful mental rotation (Liu *et al.*, 2011). A reduced need for higher-level processing may imply a bias towards early visual cortical

processing due to enhanced low-level perceptual functioning (Mottron *et al.*, 2006); a theory that is supported by results from the PPI analysis of the right cuneus seed region. On Mirror trials relative to Same trials, the ASD group increased the level of negative connectivity between the right cuneus and frontal / parietal regions, but increased the level of positive intraregional connectivity in the right cuneus. Conversely, controls showed a reduction in positive intra-regional connectivity in the right cuneus, and reduced the level of negative connectivity between right cuneus and precuneus. This pattern may indicate that, as task difficulty increases, the ASD group shows increased reliance on the early visual cortex (right cuneus) coupled with reduced recruitment of higher-level processing regions whereas controls typically reduce reliance on low-level visual perceptual processing and recruit higher level cortical regions.

Previous behavioural studies have questioned whether there is a qualitative or quantitative difference in the cognitive strategy underlying visuospatial processing mechanisms in individuals with ASD. The functional activation results from this study support the latter theory – relative to controls, the ASD group showed reduced activation in multiple brain regions during mental rotation but there were no regions in which they showed greater activity. Functional connectivity results suggest, however, that there may be both quantitative and qualitative differences in neural connectivity during mental rotation in autism. Relative to controls, the ASD group showed reduced functional connectivity between numerous brain regions indicating quantitative between-group differences. Qualitative differences were also evident. The ASD group showed connectivity to regions that did not appear to be recruited by controls – for example right caudate and temporal /cingulate regions showed connectivity in the ASD group but not controls. In addition, as discussed above, the groups showed opposing directions of connectivity between a number of regions (e.g. positive connectivity in controls but negative connectivity in the ASD group between left BA19/right BA8 and frontal regions). These findings imply qualitative between-group differences in the neural correlates of mental rotation.

There were a number of limitations to this study. Participants with ASD were limited to male, right-handed individuals with average or above average IQ. Therefore results are very specific to this group and are not representative for all individuals on the spectrum. The mental rotation paradigm did not include foils (exemplars that were neither mirror images or a rotated version of the same image). It could be argued that the participants could have employed a strategy such that if the images were not the same then they had to be correct mirror images. The PPI connectivity analysis does not measure effective connectivity – thus no causal relationships between brain regions could be identified. The study did not attempt to link functional abnormalities with structural brain abnormalities, an approach that is likely to increase understanding of the pathophysiology of ASD, however this type of integrated analysis is performed in chapters 5 and 6. A large number of neuroanatomical abnormalities in ASD have been reported in the literature. As outlined in chapter 2 (section 4.2), anatomical abnormalities have previously been reported in all brain regions that showed reduced activity in the ASD group in this study.

5. Conclusion

In the previous chapter, a striking dissociation between ASD and control groups in behavioural response times during mental rotation was reported. Given the extensive evidence for disordered neural connectivity in ASD, the aim of this study was to test the theory that connectivity abnormalities underpin atypical visuospatial processing in the disorder.

Functional connectivity analyses provided a novel insight into the neural underpinnings of the behavioural results, and, on a wider scale, into the neural mechanisms of atypical visuospatial processing in ASD. There was reduced functional connectivity between multiple brain regions during mental rotation in ASD, a finding in keeping with a large body of literature. Some connections appeared to be conserved, and were recruited in a qualitatively different way by the two groups during mental rotation in this study. Atypical visuospatial processing in ASD appeared to be associated with both quantitative and qualitative differences in functional connectivity, which may result in a combination of enhanced low-level perceptual

processing and a reduction of higher-level cortical control. Investigation of the anatomical correlates of aberrant functional connectivity is crucial to further our understanding of the pathophysiology of ASD, which is central to the approaches described in the following chapter.

4. Whole-brain analysis of white matter in ASD

1. Introduction

An accumulating body of literature suggests that autism spectrum disorders are associated with abnormal inter- and intra-regional functional connectivity. The neurobiological underpinnings of this altered functional connectivity are poorly understood. As discussed in chapter one (section 2.2), structural brain studies have identified anatomical abnormalities in ASD that could compromise connections between brain regions. The first diffusion tensor imaging study (DTI) in autism, published in 2004, reported significant disruption of white matter in the brain in ASD and since then, an increasing number of DTI studies have described alterations of white matter microstructure in the disorder.

1.1. Voxel-based analysis of DTI data in autism

The majority of the earlier DTI studies in ASD (from 2004 to 2010) used a voxel-based morphometry style of analysis that was typically used to localise differences in grey matter density. These voxel-based studies provided strong evidence for aberrant white matter in ASD, characterized by reduced Fractional Anisotropy (FA) in frontal regions (Barnea-Goraly *et al.*, 2004; Sundaram *et al.*, 2008; Cheung *et al.*, 2009; Ke *et al.*, 2009; Pardini *et al.*, 2009; Bloemen *et al.*, 2010; Noriuchi *et al.*, 2010), temporal regions (Barnea-Goraly *et al.*, 2004; Lee *et al.*, 2007b; Cheung *et al.*, 2009; Ke *et al.*, 2009; Bloemen *et al.*, 2010; Ingalhalikar *et al.*, 2010; Lange *et al.*, 2010; Noriuchi *et al.*, 2010) and the corpus callosum (Alexander *et al.*, 2007a; Keller *et al.*, 2007; Bloemen *et al.*, 2010; Noriuchi *et al.*, 2010; Shukla *et al.*, 2010a). Other studies have identified reduced organisation of white matter in the internal capsule (Keller *et al.*, 2007; Bloemen *et al.*, 2010; Shukla *et al.*, 2010a), occipital lobe (Cheung *et al.*, 2009; Bloemen *et al.*, 2010; Ingalhalikar *et al.*, 2010), anterior cingulate (Barnea-Goraly *et al.*, 2004; Noriuchi *et al.*, 2010), cingulum (Bloemen *et al.*, 2010), external capsule (Ingalhalikar *et al.*, 2010), superior longitudinal fasciculus, occipitofrontal fasciculus (Noriuchi *et al.*, 2010), middle cerebellar peduncle (Shukla *et al.*, 2010a) and insula (Ingalhalikar *et al.*, 2010).

1.2. Tract Based Spatial Statistics (TBSS)

Although these voxel-based analyses of DTI data have revealed disruption of white matter throughout the brain in autism, there is significant heterogeneity in methodologies and participants and it is difficult to identify a common underlying pattern of neuropathology across all studies (Jou *et al.*, 2011b). In addition, this typical whole-brain, voxel-by-voxel approach to analysis of diffusion-weighted data is limited by misalignment and arbitrarily applied smoothing parameters, which can produce abnormal results (Jones *et al.*, 2005; Smith *et al.*, 2006). More recently, a number of studies have used a newer approach, known as Tract-based Spatial Statistics (TBSS) (http://www.fmrib.ox.ac.uk/fsl/tbss/), that was specifically designed to resolve some of the methodological issues associated with whole-brain voxel-wise analyses of diffusion weighted data. TBSS is an automated, observer-independent method that performs voxel-wise comparisons of diffusion properties of white matter. There are a number of benefits associated with this method. It includes a method of reliable registration of subjects to a common space by projecting all subjects' FA data onto a mean FA tract skeleton. It avoids the use of a smoothing kernel. It minimizes partial volume effects and it uses permutation statistics that are not dependant on a normal distribution of data. It has been suggested that the data-driven approach of TBSS may be particularly useful in a disorder like ASD, for which regional patterns of brain abnormalities are not fully determined (Shukla *et al.*, 2011).

1.3. TBSS in autism research

To date, eight studies have used TBSS to investigate white matter microstructure in ASD. Five of these studies have reported reduced FA, increased mean diffusivity (MD) and increased radial diffusivity (RD) in various brain regions in ASD. A study of children ranging in age from 9 to 20 years revealed widespread alteration of white matter organization characterized by reduced FA and increased MD and RD in regions of the anterior and posterior limb of the internal capsule, the inferior longitudinal fasciculus (ILF), inferior fronto-occipital fasciculus (IFOF), superior longitudinal fasciculus (SLF), corpus callosum, cingulum, anterior thalamic

radiation and corticospinal tract (Shukla *et al.*, 2011). A similar study of children with ASD, aged 8 to 18 years, showed some overlap with this study; regions of the IFOF, SLF, corpus callosum and superior corona radiata were found to have impaired organization of white matter, characterized by increased MD and RD (Ameis *et al.*, 2011). Another study of children with ASD, aged between 5 and 17, identified reductions in FA in multiple association, commissural, and projection tracts; the most severely affected tracts again included the IFOF, SLF and forceps minor of the corpus callosum (Jou *et al.*, 2011b). Reduced FA and increased MD were reported in the corpus callosum as well as in the right anterior thalamic radiation and left uncinate fasciculus in children with ASD aged between 8 and 14 years (Cheon *et al.*, 2011). Interestingly, unaffected siblings of children with autism were also found to have reduced FA and AD in white matter of frontal, parietal and temporal lobes relative to controls and it has been suggested that "white matter structure may represent a marker of genetic risk for autism or vulnerability to development of this disorder" (Barnea-Goraly *et al.*, 2010).

In contrast to these findings of reduced FA, three other studies using TBSS have reported *increases* in FA in various regions of white matter. In a study of very young (<4 years old) children with autism, increased FA and reduced RD were reported in the genu and midbody of the corpus callosum, left SLF and right and left cingulum (Weinstein *et al.*, 2011). This finding of increased FA is in contrast with the majority of previous studies and although it may be related to the young age of the participants (Wolff *et al.*, 2012), two other TBSS studies of older children have also found increased FA: increased FA (and reduced RD) have been reported in the frontal lobe, right cingulate gyrus, bilateral insula, right superior temporal gyrus and bilateral middle cerebellar peduncle (Cheng *et al.*, 2010) and in areas of the optic radiation and right IFOF (Bode *et al.*, 2011).

Taken together, these TBSS studies suggest that white matter organization is disrupted in numerous major white matter tracts in ASD, but despite the homogeneity of the TBSS analytic method and the relative similarity of participants in the majority of these studies, results do not provide a clear

picture of common underlying neuropathology. It is possible that the discrepancies in results from these eight TBSS studies are a consequence of the use of different DTI acquisition protocols and pre-processing methodologies (Keedwell *et al.*, 2012).

In terms of DTI acquisition protocols, it is known that FA values are affected by the number of diffusion-weighting directions (N): in brain regions of high anisotropy, FA values increase with increased N but conversely in brain regions of low anisotropy, FA values reduce with increased N (Giannelli *et al.*, 2010). There is significant variation of FA with N and importantly the level of this variation is comparable to the anisotropy change seen in some clinical disorders. Therefore it has been suggested that in clinical studies, the number of diffusion weighting directions may "represent a confounding variable for anisotropy measurements" (Giannelli *et al.*, 2010) and a minimum N of >20 is recommended. It is noteworthy that five of the eight previous TBSS studies in ASD used fewer than 20 diffusion weighting directions (Barnea-Goraly *et al.*, 2010; Cheng *et al.*, 2010; Ameis *et al.*, 2011; Shukla *et al.*, 2011; Weinstein *et al.*, 2011).

With regard to pre-processing methodologies, typical pre-processing of diffusion data involves correcting for eddy current induced distortions and for head motion. When correcting for subject motion in DTI data, it is important when realigning the images to reorient the B matrix so that orientational information is correctly preserved (Leemans and Jones, 2009). Neglecting to orient the B matrix introduces a significant bias in diffusion measures yet many software packages do not offer this rotational step (Leemans and Jones, 2009). None of the eight previous studies using TBSS in ASD mention B matrix reorientation in their methods.

Artifacts due to cardiac pulsation do not appear to have been corrected for in previous TBSS analyses in ASD. Cardiac pulsation commonly leads to artifact in clinical DTI acquisitions. Cardiac gating is a technique used to prevent distortion of DTI images caused by cardiac pulsation whereby scans are only taken during diastole, when arterial blood flow is minimal. The difficulty with cardiac gating is that it results in longer acquisition times and therefore it is not often used in clinical DTI studies. It has been shown,

however, that if the diffusion tensor is modeled with the commonly used least squares regression method and fails to account for signal artifact introduced by cardiac pulsation, a single corrupted data point in the diffusion dataset can significantly affect the values of the computed diffusion tensor values (Chang *et al.*, 2005). RESTORE (Robust Estimation of Tensors by Outlier Rejection) is a pre-processing algorithm for DTI data that offers an effective approach to obtain diffusion tensor parameters immune from cardiac induced artifacts when data are acquired with ungated acquisition protocols (Chang *et al.*, 2005). The RESTORE algorithm has been implemented in at least one software package (ExploreDTI, (Leemans *et al.*, 2009)). In the eight TBSS studies in ASD, none used cardiac gating, nor did any mention using the RESTORE algorithm for pre-processing. Data in these studies are therefore susceptible to significant signal artifact introduced by cardiac pulsation.

1.4. Aims and hypothesis

In summary, differences in acquisition protocols, failure to reorient the B matrix and failure to correct for cardiac pulsation may contribute to the differences reported in diffusion measures in eight previous TBSS studies in ASD. The aim of this study was therefore to analyse an optimally pre-processed, high angular resolution DTI dataset with TBSS to investigate whether there are true differences in diffusion measures between participants with ASD and controls. Based on the literature, we hypothesized that there would be alterations of white matter microstructure in ASD, in particular in white matter of the IFOF, SLF and corpus callosum.

2. Methods

2.1. Participants

Twenty-five right-handed male individuals with ASD (mean age 17.28, mean IQ 106.84) and twenty-five right-handed age and IQ-matched male neurotypical controls (mean age 17.37, mean IQ 110.72) were included in the analysis. For both age and IQ there were no significant group differences, $p > 0.05$ (see Table 1). Ascertainment and diagnosis of participants is described fully in chapter 2, section 2.1. All fifty participants

who completed the fMRI scan session had a diffusion weighted MRI (DW-MRI) scan. Data from all fifty DW-MRI scans were analysed.

Table 1: Demographics of study participants

	Control	ASD	p-value
Number	25	25	
Gender	Male (25)	Male (25)	
Age Mean (SD, Range)	17.37 (2.67, 13.6-21.3)	17.28 (2.87, 13.0-21.8)	0.94
Full scale IQ* Mean (SD, Range)	110.72 (16.02, 84-147)	106.84 (14.54, 84-145)	0.37
Verbal IQ^ Mean (SD, Range)	112.9 (16.88, 81-130)	104 (18.32, 79-130)	0.26
Performance IQ- Mean (SD, Range)	120.09 (25.14, 91-155)	122.68 (17.40, 99-155)	0.78
Handedness	Right (25)	Right (25)	
Medication	None	None	
Ethnicity	Irish (25)	Irish (25)	

*Full scale IQ was estimated based on four sub-scales of the WISC/WAIS (Wechsler Intelligence Scale for Children (WISC-III or IV UK), (Wechsler, 1991, 2004) and Wechsler Adult Intelligence Scale (WAIS-III), (Wechsler, 1997).

^Verbal IQ was estimated using the Information and Vocabulary subtests of the WISC III for n=10 participants with ASD and n=10 matched controls, using Sattler's method (Sattler, 1992). It was not possible to produce a Verbal IQ for n=12 participants with ASD and n=12 controls, as the WISC IV was used to estimate full scale IQ for these participants.

~ Performance IQ was estimated using the Picture Completion and Block Design subtests of the WISC III for n=10 participants with ASD and n=10 for controls, using Sattler's method (Sattler, 1992). It was not possible to produce a Performance IQ for n=12 participants with ASD and n=12 controls, as the WISC-IV was used to estimate full scale IQ for these participants.

2.2. Data acquisition

Whole-brain High Angular Resolution Diffusion Imaging (HARDI) data were acquired from the 25 participants with ASD and 25 age- and IQ-matched typically developing controls on a Philips Intera Achieva 3.0 Tesla MR system (Best, Netherlands) equipped with an eight-channel head coil. A parallel Sensitivity Encoding (SENSE) approach (Pruessmann *et al.*, 1999) with a reduction factor of 2 was utilized for all diffusion weighted image acquisitions. Single shot spin echo-planar imaging was used to aquire

diffusion weighted data using the following parameters: echo time (TE) 79ms, repetition time (TR) 20122ms, field of view 248mm, matrix 128, isotropic voxel resolution 2x2x2mm, 65 slices with 2mm thickness with no gap between slices. Diffusion gradients were applied in 61 isotropically distributed orientations with b=1500s/mm2. Four images with b=0 s/mm2 were also acquired for the purpose of registration. Total scan time was 24.3 minutes.

2.3. Data analysis

2.3.1. Pre-processing of DW-MRI data

All pre-processing and tractography analysis was performed with the diffusion MR toolbox ExploreDTI (http://www.ExploreDTI.com) (Leemans *et al.*, 2009). Each DW-MRI dataset was corrected for eddy current induced geometric distortions and subject motion by realigning all diffusion weighted images (DWIs) to the b=0 images using elastix (Klein *et al.*, 2010), with an affine co-registration technique (with 12 degrees of freedom) and mutual information as the cost function (Pluim *et al.*, 2003). In this procedure, motion distortion correction was applied taking into account the B-matrix orientation (Leemans and Jones, 2009) and the tensor model was fitted to the data using the RESTORE approach (Chang *et al.*, 2005), which uses a process of iteratively reweighted least-square regression for outlier identification and subsequent removal thus minimising artifacts such as cadiac pulsation and subject motion. Motion and distortion corrected FA, MD, AD and RD images were extracted from the pre-processed data.

2.3.2. Tract-based spatial statistics (TBSS)

Voxelwise statistical analysis of the FA data was carried out using TBSS (Tract-Based Spatial Statistics) (Smith *et al.*, 2006), part of FSL (Smith *et al.*, 2004). First, the FA images created during the pre-processing step in ExploreDTI were brain-extracted using BET (Smith, 2002). All participants' FA data were then aligned into a common space using the nonlinear registration tool FNIRT, which uses a b-spline representation of the registration warp field (Rueckert *et al.*, 1999). Next, the mean FA image was created and thinned to generate a mean FA skeleton, which represents the

centres of all tracts common to the group. Each subject's aligned FA data were then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistics. To apply TBSS to the MD, AD and RD data, the tbss_non_FA tool in FSL was used. This applies the non-linear warps and skeleton projection that were achieved using the FA images to the MD, AD and RD images.

2.3.3. Statistical analysis

Group differences in FA, MD, AD, and RD were investigated using a voxel-wise non-parametric rank order Brunner-Munzel (Brunner and Munzel, 2000) test within the NPM (Non-Parametric Mapping) software package (Rorden *et al.*, 2007) (http://www.mcauslandcenter.sc.edu/mricro/mricrom/index.html). Parametric statistical tests make a number of assumptions regarding the distribution of the data whereas the use of a non-parametric equivalent test makes no such assumption about the data. The Brunner-Munzel test has been shown to be an appropriate test statistic in datasets with group sizes greater than 9 per group (Medina *et al.*, 2010), as is the case in this study with N=25 for each group. Voxel-wise FA / MD / AD / RD differences were corrected for multiple comparisons using a false discovery rate (FDR) (Genovese *et al.*, 2002) threshold of 5%. Standard procedures for multiple correction testing (e.g. Bonferroni), often prove to be too stringent with very strong Type 1 error correction and usually require data to be smoothed for optimum performance [as performed in familywise error (FWE)]. FDR is a data-driven approach that works best on unsmoothed data, therefore it is an ideal method for analysis of data from TBSS (Genovese *et al.*, 2002).

The anatomic location of each cluster that showed a significant between-group difference in FA / MD / AD / RD was determined using the atlas tool in FSLView (http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html), which incorporates a number of anatomic templates including the Harvard-Oxford Cortical and Subcortical Structural Atlases, Juelich Histological Atlas, Johns Hopkins University DTI-based WM atlases, Talairach atlas, MNI structural atlas and Oxford thalamic connectivity atlas.

3. Results

3.1. Voxelwise TBSS analysis

Voxel-wise TBSS analysis revealed a number of small clusters that showed significant reductions in FA and AD and significant increases in MD in the ASD group relative to controls. There were no between-group differences in RD on voxel-wise TBSS analysis.

3.1.1. FA

Relative to controls, individuals with ASD showed reduced FA in white matter in brain regions including: (1) left precentral gyrus within the left SLF, (2) left superior temporal gyrus in the left SLF, (3) left precentral gyrus within the left corticospinal tract, (4) right and (5) left anterior thalamic radiation and (6) left middle frontal gyrus (see Table 2a and Figure 1). There were no brain regions in which FA was greater in the ASD group than in the control group.

3.1.2. MD

Individuals with ASD showed increased MD in white matter of a number of major association and interhemispheric tracts including: (1) right SLF (in right middle and inferior frontal gyri), (2) right ILF (in right middle occipital gyrus), (3) left optic radiation (in left visual cortex BA18), and (4) forceps major (in right visual cortex BA17). Increased MD in the ASD group was also present in white matter of the right superior frontal gyrus, right middle frontal gyrus (in four separate clusters), left middle temporal gyrus and left cuneus (see Table 2b and Figure 1). There were no brain regions in which MD was lower in the ASD group than in the control group.

3.1.3. AD

AD was reduced in the ASD group relative to controls in white matter of the (1) left IFOF and left optic radiation (in the temporal lobe), (2) left corticospinal tract (in the left precentral gyrus) and (3) right optic radiation (in the right thalamus) (see Table 2c and Figure 1). There were no brain regions showing increased AD in the ASD group relative to controls.

White matter tract	Corresponding cortical location	Peak intensity voxel location (MNI co-ords)			Mean (SD) ASD	Mean (SD) Control	F - value	Cluster size (voxels)
		x	y	z				
FA								
L SLF	L precentral gyrus, frontal lobe	-37	-1	24	0.37 (0.01)	0.404 (0.03)	24.76	5
L CST	L precentral gyrus, frontal lobe	-31	-12	52	0.427 (0.05)	0.491 (0.05)	22.86	2
R ATR	R thalamus	9	-21	11	0.146 (0.01)	0.163 (0.01)	19.58	2
L SLF	L superior temporal gyrus	-47	-33	5	0.385 (0.06)	0.460 (0.07)	18.58	2
N/A	L middle frontal gyrus	-27	36	37	0.150 (0.02)	0.170 (0.01)	19.22	1
L ATR	L thalamus	-1	-11	4	0.309 (0.11)	0.457 (0.11)	22.31	1

Table 2a: Voxelwise TBSS analysis results. Location and size of clusters showing significant between-group differences in FA. (Abbreviations L: Left, R: Right, SLF: Superior Longitudinal Fasciculus, CST: Corticospinal tract, ATR: Anterior thalamic radiation, N/A: No specific white matter tract at this location).

White matter tract	Corresponding cortical location	Peak intensity voxel location (MNI co-ords)			Mean (SD) ASD	Mean (SD) Control	F	Cluster size (voxels)
		x	y	z				
MD (mm^2/sec)								
R SLF	R middle frontal gyrus	34	8	33	0.000690 (0.00004)	0.000638 (0.00003)	25.15	6
R SLF	R inferior frontal gyrus	45	7	14	0.000545 (0.00002)	0.000521 (0.00002)	13.95	2
R ILF	R middle occipital gyrus	37	-77	10	0.000390 (0.00002)	0.000367 (0.00002)	17.73	2
N/A	R middle frontal gyrus	38	7	37	0.000356 (0.00002)	0.000346 (0.00002)	12.70	1
N/A	R middle frontal gyrus	36	14	34	0.000734 (0.00003)	0.000692 (0.00002)	29.27	1
N/A	R middle frontal gyrus	31	30	23	0.000740 (0.00004)	0.000701 (0.00004)	12.01	1
L OR	L cuneus BA18	-10	-85	21	0.000793 (0.00005)	0.000735 (0.00003)	25.46	1
F maj	R cuneus BA17	14	-91	13	0.000705 (0.00004)	0.000663 (0.00003)	19.97	1
N/A	L cuneus BA18	-30	-93	0	0.000676 (0.00003)	0.000636 (0.00004)	15.81	1
N/A	R middle frontal gyrus	40	52	-8	0.000741 (0.00006)	0.000668 (0.00007)	16.27	1
N/A	L middle temporal gyrus	-50	-20	-9	0.000746 (0.00009)	0.000675 (0.00004)	14.13	1

Table 2b: Voxelwise TBSS analysis results. Location and size of clusters showing significant between-group differences in MD. (Abbreviations L: Left, R: Right, SLF: Superior Longitudinal Fasciculus, ILF: Inferior Longitudinal Fasciculus OR: Optic radiation, F maj: Forceps major, N/A: No specific white matter tract at this location).

White matter tract	Corresponding cortical location	Peak intensity voxel location (MNI co-ords)			Mean (SD) ASD	Mean (SD) Control	F	Cluster size (voxels)
		x	y	z				
AD (mm^2/sec)								
L IFOF L OR	Temporal lobe	-27	-60	23	0.000962 (0.00007)	0.001071 (0.00009)	23.96	4
L CST	L precentral gyrus	-30	-11	51	0.000697 (0.00007)	0.000797 (0.00009)	18.64	3
R OR	R thalamus	26	-28	-1	0.001174 (0.00008)	0.001294 (0.00011)	17.64	3

Table 2c: Voxelwise TBSS analysis results. Location and size of clusters showing significant between-group differences in AD. (Abbreviations L: Left, R: Right, CST: Corticospinal tract, , OR: Optic radiation, IFOF: Inferior fronto-occipital fasciculus).

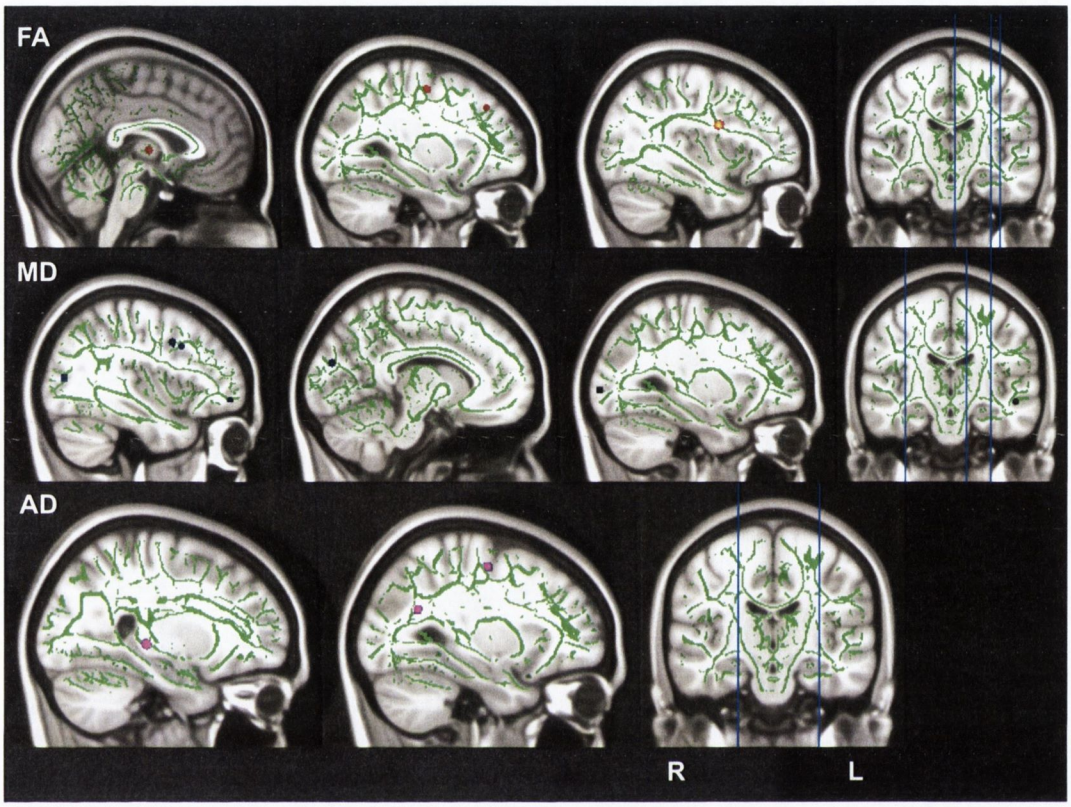


Figure 1: Sagittal brain sections illustrating the results of the voxelwise TBSS analysis. Red-yellow clusters indicate a selection of regions showing significantly reduced FA in the ASD group on TBSS analysis; blue clusters indicate a selection of the regions showing significantly increased MD in the ASD group and violet clusters indicate the regions showing reduced AD in the ASD group. Blue lines on coronal slices illustrate the locations of the sagittal slices that are shown.

4. Discussion

Using an optimized DTI acquisition protocol and pre-processing methods, Tract Based Spatial Statistics revealed that there was significant disruption of white matter microstructural organization in small clusters of a number of major white matter tracts in individuals with ASD. To minimize the possibility of between-group differences arising from error, this study used a high angular resolution diffusion imaging (HARDI) acquisition protocol, and diffusion data was pre-processed using advanced methods to ensure that high quality images were input into the TBSS model.

Relative to controls, the ASD group showed significantly reduced FA, reduced AD and increased MD in small clusters of white matter of bilateral SLF, right ILF, left IFOF, left corticospinal tract, bilateral anterior thalamic radiation, bilateral optic radiation and forceps major. Despite the small volume of the clusters, it is reasonable to assume that brain regions identified in this study reflect locations of genuine white matter pathology in the ASD group. The regions of disrupted white matter in this study are similar to those reported in previous TBSS studies (Ameis *et al.*, 2011; Cheon *et al.*, 2011; Jou *et al.*, 2011b; Shukla *et al.*, 2011), they are biologically plausible and the study used optimal acquisition and pre-processing methodology. Methodological research published since this TBSS analysis was performed has, however, highlighted some limitations with the TBSS approach that could potentially lead to TBSS results that are less reliable than previously thought and these are discussed in detail in the 'Limitations' section of this Discussion.

Assuming that the results of this study do reflect true abnormalities of white matter microstructure in the ASD group, it is important to understand how disrupted white matter organisation in these regions might impact on cognitive functioning in ASD. The first section of this Discussion considers the roles of the white matter tracts that are implicated by the results of this study and the second section discusses the functional implications of these results.

4.1. Roles of white matter tracts implicated by study results

4.1.1. Superior longitudinal fasciculus

Regions of both right and left SLF showed abnormal microstructural organization in this study. FA was reduced in the ASD group in two regions of the temporal part of the left SLF, the arcuate fasciculus. The arcuate fasciculus in the left hemisphere links Broca's and Wernicke's language areas and is known to be of great importance in language. Previous functional neuroimaging work has demonstrated reduced functional connectivity between language areas during sentence comprehension in ASD (Just *et al.*, 2004) and it has been suggested that the language impairment in autism may be associated with structural abnormalities of the white matter in the arcuate fasciculus (Fletcher *et al.*, 2010). Findings of reduced FA in the arcuate fasciculus in this study add to a growing number of reports of abnormal microstructure of white matter in this tract (Fletcher *et al.*, 2010; Kumar *et al.*, 2010; Ameis *et al.*, 2011; Jeong *et al.*, 2011; Jou *et al.*, 2011b; Lo *et al.*, 2011; Shukla *et al.*, 2011) and are in keeping with a theory of abnormal neural connectivity in ASD (Just *et al.*, 2004). The role of the right SLF is less well documented, but it is connected to the superior temporal sulcus, a brain region important for biological motion processing, which is abnormal in ASD (Kaiser *et al.*, 2010). MD was increased in the ASD group in two frontal regions of the right SLF in this study, again adding to literature that has documented disruption of white matter in this tract in ASD (Barnea-Goraly *et al.*, 2010; Cheng *et al.*, 2010; Kumar *et al.*, 2010; Shukla *et al.*, 2011).

4.1.2. Inferior fronto-occipital fasciculus

The IFOF is an extensive long-range association tract in the human brain linking frontal, parietal, temporal and occipital lobes (Martino *et al.*, 2010). The function of the IFOF is not well understood, but it is thought to play a role in visual processing, reading, attention and the semantic system (Catani and Mesulam, 2008; Doricchi *et al.*, 2008; Epelbaum *et al.*, 2008; Fox *et al.*, 2008; Rudrauf *et al.*, 2008). The right IFOF has also been associated with emotion recognition (Philippi *et al.*, 2009), which is of particular relevance for autism research given the extensive evidence for impaired emotion

recognition in the disorder. Previous TBSS and tractography studies have reported structural abnormalities of the IFOF in ASD (Ameis *et al.*, 2011; Jou *et al.*, 2011b; Shukla *et al.*, 2011; Thomas *et al.*, 2011). In this study, the right IFOF was unaffected, but the ASD group showed reduced AD of the left IFOF in a cluster in the temporal lobe.

4.1.3. Inferior longitudinal fasciculus

The ILF is an association tract linking occipital and temporal lobes of the brain. It has a role in visual perception (ffytche and Catani, 2005; Ffytche, 2008), face recognition (Fox *et al.*, 2008), reading (Epelbaum *et al.*, 2008), visual memory (Ross, 2008) and other functions related to language (Catani and Mesulam, 2008; Catani and Thiebaut de Schotten, 2008). Consistent with several DTI studies that have reported abnormalities of the ILF in ASD (Pugliese *et al.*, 2009; Shukla *et al.*, 2011; Thomas *et al.*, 2011; Wolff *et al.*, 2012), individuals with ASD in this study showed altered white matter structure in the right ILF characterized by an increase in MD.

4.1.4. Anterior thalamic radiation

The anterior thalamic radiation is a major white matter projection from the thalamus that carries reciprocal projections between the thalamus, prefrontal cortex and striatum, which participate in prefrontal-subcortical circuits (Behrens *et al.*, 2003). This tract is likely to play an important role in processing speed and executive functioning (Tekin and Cummings, 2002; Van der Werf *et al.*, 2003). Thalamic abnormalities have been implicated in the pathophysiology of autism [see (Cheon *et al.*, 2011) for review] and abnormal functional connectivity between the thalamus and various brain regions has been reported in autism (Mizuno *et al.*, 2006; Kleinhans *et al.*, 2008; Mostofsky *et al.*, 2009). In this study, participants with ASD showed reduced FA in both the right and left anterior thalamic radiation, in the region of right and left thalamus, a finding in keeping with a number of DTI studies (Cheung *et al.*, 2009; Tan *et al.*, 2010; Cheon *et al.*, 2011; Shukla *et al.*, 2011).

4.1.5. Forceps major

The forceps major links left and right occipital lobes through the splenium and appears to play an important role in visual perception, manipulation of visuospatial information, memory and topographical orientation (Rudge and Warrington, 1991; Tamura *et al.*, 2007). In this study, participants with autism showed increased MD in the forceps major in a region of the right visual cortex. This result is similar to that reported by one previous DTI tractography study (Hong *et al.*, 2011), and it is interesting to note that the two study populations were quite similar. Other DTI studies have shown mixed results; two studies reported abnormalities in the forceps major (Ameis *et al.*, 2011; Jou *et al.*, 2011b) but three others did not find abnormalities of white matter in this tract (Thomas *et al.*, 2011; Weinstein *et al.*, 2011; Wolff *et al.*, 2012).

4.2. Functional implications of disrupted white matter microstructure

In the literature, a typical interpretation of reduced FA, reduced AD, increased MD or increased RD is that there is reduced "integrity" of white matter. A reduction in FA is thought to be associated with disrupted organisation of white matter fibre tracts and may reflect demyelination, axonal damage or loss of white matter coherence (Basser, 1995; Basser and Pierpaoli, 1996; Werring *et al.*, 2000; Alexander *et al.*, 2007b). Reduced axial diffusivity may indicate altered axonal integrity. Increased radial diffusivity is considered to be a sensitive marker for demyelination (Song *et al.*, 2005) and an increase in mean diffusivity is thought to reflect oedema, demyelination and axonal loss [see (Alexander *et al.*, 2007b) for review].

There is a growing awareness, however, that differences in these tensor-derived measures need to be interpreted cautiously. It has long been recognised that the diffusion tensor model is unable to describe the diffusion correctly in regions of complex fibre architecture, (e.g. crossing, kissing, bending, splaying fibres) because the tensor model cannot resolve more than a single axon direction within each voxel (Frank, 2001; Alexander *et al.*, 2002; Frank, 2002; Tuch *et al.*, 2002). In such crossing fibre regions, tensor-derived measures such as FA, AD, RD and MD are unreliable; FA and MD

values are lower compared with their values in single-fibre regions and the interpretation of AD and RD measures is complex (Wheeler-Kingshott and Cercignani, 2009; Jones and Cercignani, 2010; Jeurissen *et al.*, 2012; Vos *et al.*, 2012). In regions of white matter with more than one fibre orientation, an increase or decrease in FA or MD may correspond to increased or decreased integrity of white matter, but alternatively it may simply arise from changes in the relative volume fractions of various fibre populations (Jeurissen *et al.*, 2012; Vos *et al.*, 2012). Until recently, it was thought that the prevalence of crossing fibres in white matter was approximately 33% (Behrens *et al.*, 2007), however a recent study has estimated the proportion of white matter voxels containing crossing fibres to be at least 90% (Jeurissen *et al.*, 2012). This unexpectedly high prevalence of crossing fibres in white matter now makes the interpretation of increases and decreases in tensor-derived scalar measures highly ambiguous. According to Jeurissen et al (2012), the proportion of white matter where tensor-derived measures are expected to be significantly confounded is 75%, therefore as "the interpretation commonly ascribed to these measures is valid only in a single-fibre population, this implies that there are very few regions of brain white matter where the measures can reliably be interpreted as markers of white matter integrity" (Jeurissen *et al.*, 2012).

Given these recent findings, it is difficult to interpret the impact that the reduced FA and AD and increased MD found in this study have on the structural "integrity" of white matter. The study has simply shown that at a voxel-wise level, microstructural organization of white matter differs between groups in specific regions of a number of white matter tracts. There is no information relating to the overall microstructural organization of the entire tract. This information is vital when investigating whether long-range structural connectivity is impaired in ASD. Indeed, in a paper describing the protocol for TBSS, the authors emphasised that TBSS should not be used "as a replacement for careful tractography-based connectivity analysis of diffusion data, which should ideally be used in conjunction with a voxelwise analysis" (Smith *et al.*, 2007) (page 500).

4.3. Limitations of TBSS

As mentioned in the introduction to this discussion, although TBSS is a widely used analytical approach for whole-brain DTI analysis, very recent methodological research has revealed a number of significant limitations of this method. Even though this study used optimal acquisition and pre-processing methods in a well-characterized group of individuals with ASD, it appears that the TBSS analytic method itself has the potential to introduce error in results.

First, it has recently been shown that the statistical sensitivity of the FA "skeleton" used in the TBSS method is not optimal (Edden and Jones, 2011). In order to achieve reliable registration of participants' DTI data to a common space, TBSS generates a mean FA "skeleton", which represents the centres of all tracts common to the group. Each participant's FA data is then projected onto this skeleton. Two key observations of this study were that the variance of diffusion metrics is non-uniform across the skeleton (with more central structures having less variance within a population) and that skeleton thickness depends on the relative orientation of the imaging matrix. These findings have significant implications on the interpretation of results obtained from TBSS analysis; for example structures that are obliquely oriented to the imaging matrix are more likely to show significant differences than when aligned with the imaging matrix (Edden and Jones, 2011).

Second, TBSS may be underpowered to detect significant between-group differences with small samples and/or subtle effects (Cykowski et al, 2011). A study investigating cingulum white matter in young women at risk of depression highlights this issue (Keedwell *et al.*, 2012). Using diffusion tractography, reduced FA was detected in bilateral cinguli of healthy adults with a family history of depression relative to controls. Correlations between FA in the cingulum tracts and behavioural measures were also demonstrated. TBSS analysis of the same data, however, failed to find any significant group differences or correlations in any of the metrics studied. The authors offer an explanation for the difference between TBSS and tractography results, and based on previous work (Kanaan *et al.*, 2006),

suggest that "taking an average value along a tract... may be a more sensitive approach than taking highly localized – and independent – measurements along the tract" (page 5, in press) (Keedwell et al., 2012). Keedwell and colleagues argue that a tractography based approach may increase power to detect group effects over the TBSS approach (Keedwell *et al.*, 2012).

Third, an important study investigating the stability of the TBSS method has demonstrated that in a 'literature-typical' sample size of 20 participants, results from TBSS are quite unstable. The study explored stability using a bootstrapping method [100 permutations, 24 participants (omitting 4)] and showed that significant white matter tracts were very different among permutations (Bells *et al.*, 2012). The sample size was very similar to that used in this study, and the DTI acquisition protocol and pre-processing algorithm were almost identical. This may indicate that TBSS results from this ASD study may not be as reliable as was initially thought.

In summary, recent methodological research has revealed that there are significant limitations of the TBSS approach to DTI analysis. Even though this study used optimal acquisition and pre-processing methods at the time of data collection, in a well-characterized group of individuals with ASD, it appears that the TBSS analytic method may introduce error in results. It has been suggested that a diffusion tractography approach is likely to provide more reliable and readily interpretable information about "structural connectivity" in ASD (Jeurissen *et al.*, 2012). Diffusion tractography is an analytic approach whereby white matter bundles are isolated in vivo in the human brain using fibre-tracking software on DTI data. Diffusion measures extracted from the tract can then be used to give an estimate of the overall level of white matter organization within this tract and a level of anatomical 'connectivity' can be inferred from this. In the next chapter, such a tractography analysis of this data is used.

5. Conclusion

This study used high quality diffusion MRI data with TBSS to investigate voxel-wise differences in white matter organization between individuals with

ASD and neurotypical controls. It was hoped that this approach would help to clarify the rather heterogenous pattern of results of previous TBSS studies in ASD by identifying brain regions that showed genuine disruption of white matter. Relative to controls, the ASD group showed small clusters of significantly reduced FA and AD and increased MD in white matter in a number of brain regions. Recent methodological research has demonstrated that there are two difficulties with interpretation of these results; firstly the interpretation of tensor-derived diffusion metrics is ambiguous and secondly the TBSS method may not be reliable in sample sizes similar to this study. It is therefore difficult to determine the functional significance of the findings of this TBSS study. Isolation of complete white matter tracts using diffusion tractography is likely to provide more readily interpretable information about structural connectivity in ASD.

5. Constrained Spherical Deconvolution based Tractography in ASD

1. Introduction

Autism and autism spectrum disorders (ASDs) are associated with a marked disturbance of neural functional connectivity. As discussed in detail in chapters 1 and 3, a growing body of literature suggests that functional connectivity abnormalities in ASD are widespread, affect multiple cognitive functions and can be characterized by both reduced [e.g. (Just *et al.*, 2004)] and increased [e.g. (Monk *et al.*, 2009)] patterns of connectivity. The pathophysiology of this abnormal functional connectivity is poorly understood, but it may arise from disrupted organisation of white matter in inter and intra-hemispheric tracts.

1.1. Diffusion tractography in autism

Diffusion weighted (DW) MRI allows non-invasive, in vivo examination of white matter tissue microstructure in the human brain. There has been a recent explosion of interest in using fibre-tracking on DW-MRI data to investigate brain connectivity in both healthy and disordered populations. Over the past four years, a number of studies have used diffusion tensor tractography to isolate specific white matter tracts in autism. As outlined in chapter 1 section 2.2.3, quantitative analysis of the diffusion parameters extracted from these tracts have revealed that white matter integrity is disrupted in autism in the corpus callosum (CC) (Kumar *et al.*, 2010; Hong *et al.*, 2011; Jeong *et al.*, 2011; Lo *et al.*, 2011; Thomas *et al.*, 2011; Weinstein *et al.*, 2011; Wolff *et al.*, 2012), uncinate fasciculus (UF) (Pugliese *et al.*, 2009; Kumar *et al.*, 2010; Jeong *et al.*, 2011; Lo *et al.*, 2011; Poustka *et al.*, 2011; Thomas *et al.*, 2011; Wolff *et al.*, 2012), cingulum (Pugliese *et al.*, 2009; Kumar *et al.*, 2010; Lo *et al.*, 2011; Weinstein *et al.*, 2011), arcuate fasciculus (AF) (Fletcher *et al.*, 2010; Kumar *et al.*, 2010; Jeong *et al.*, 2011; Lo *et al.*, 2011; Wan *et al.*, 2012), superior longitudinal fasciculus (Verhoeven *et al.*, 2011) inferior longitudinal fasciculus (ILF) (Pugliese *et al.*, 2009; Thomas *et al.*, 2011; Wolff *et al.*, 2012), fornix (Wolff *et al.*, 2012) but see (Poustka *et al.*, 2011), inferior fronto-occipital fasciculus (IFOF)

(Thomas *et al.*, 2011) but see (Pugliese *et al.*, 2009; Kumar *et al.*, 2010), anterior thalamic radiation, internal capsule (Wolff *et al.*, 2012), short intracerebellar fibres (Catani *et al.*, 2008), hippocampo-fusiform and amygdalo-fusiform pathways (Conturo *et al.*, 2008), fronto-fusiform and fronto-temporal tracts (Sahyoun *et al.*, 2010b) and fronto-striatal tracts (Langen *et al.*, 2012).

1.2. Problems with diffusion tensor tractography

The diffusion tensor model is currently the most commonly used framework to relate the diffusion signal to the direction of the fibres (Basser *et al.*, 1994; Mori and van Zijl, 2002) and diffusion tensor tractography is now a widely used method for assessing white matter microstructure and connectivity. In voxels containing more than one coherently oriented fibre population (e.g. crossing fibres) it is well recognized that the diffusion tensor model is inadequate (Wedeen *et al.*, 2000; Alexander *et al.*, 2002; Frank, 2002; Tuch *et al.*, 2002; Tournier *et al.*, 2011). Such voxels containing crossing fibres occur frequently throughout the white matter due to partial volume effects between adjacent tracts (Tournier *et al.*, 2008; Vos *et al.*, 2011). As discussed in chapter 4 (section 4.2), a recent paper revealed the scale of this problem to be much more significant than previously thought; in a study that was specifically designed to investigate the extent of crossing fibres in white matter, the proportion of white matter voxels containing crossing fibres was estimated to be at least 90% (Jeurissen *et al.*, 2012). This value is far higher than the 33% that had previously been reported (Behrens *et al.*, 2007) and has profound implications for diffusion tensor tractography and tensor-derived scalar measures (Jeurissen *et al.*, 2012; Vos *et al.*, 2012). In regions of complex fibre architecture, tensor derived measures such as FA and MD are unreliable (Jones and Cercignani, 2010; Vos *et al.*, 2012) and the interpretation of these measures is highly ambiguous (Jeurissen *et al.*, 2012). In addition, in voxels with crossing fibres the diffusion tensor model cannot adequately describe fibre orientation, thus the underlying orientation of the white matter tracts cannot be reliably estimated. Therefore, fibre-tracking algorithms that rely on the diffusion tensor will produce unreliable results in regions containing multiple fibre orientations. This may result in a

failure to identify real white matter connections – for example where tracking terminates prematurely (Behrens *et al.*, 2007; Jeurissen *et al.*, 2011). Alternatively it may lead to the isolation of white matter connections that do not really exist – where tracking switches to an unrelated adjacent tract (Pierpaoli *et al.*, 2001; Jeurissen *et al.*, 2011).

The unexpectedly high prevalence of crossing fibres in white matter is likely to have significant implications for diffusion tensor imaging in clinical research. For example, fifteen of the sixteen studies that have investigated white matter tracts in ASD have used DTI and tensor-modelled tractography methods (Catani *et al.*, 2008; Conturo *et al.*, 2008; Pugliese *et al.*, 2009; Fletcher *et al.*, 2010; Kumar *et al.*, 2010; Sahyoun *et al.*, 2010b; Hong *et al.*, 2011; Jeong *et al.*, 2011; Jou *et al.*, 2011a; Poustka *et al.*, 2011; Thomas *et al.*, 2011; Weinstein *et al.*, 2011; Langen *et al.*, 2012; Wan *et al.*, 2012; Wolff *et al.*, 2012) while one study used diffusion spectrum imaging (Lo *et al.*, 2011). Given the inadequacies of the diffusion tensor model in regions of crossing fibres, it is possible that the tensor derived scalar measures and tractography results that have been reported in these studies are unreliable.

1.3. Constrained spherical deconvolution

A number of more complex diffusion reconstruction schemes have been proposed to overcome the limitations associated with the DTI model. One such approach is that of constrained spherical deconvolution (CSD), a method that allows reliable estimation of fibre orientation in the presence of intra-voxel orientational heterogeneity (Tournier *et al.*, 2004; Tournier *et al.*, 2007; Tournier *et al.*, 2008). The CSD method assumes that the measured diffusion MR signal is comprised of the spherical convolution of the response function (the diffusion weighted signal for a typical fibre population) with the fibre orientation distribution (FOD). Therefore the FOD can be estimated by spherically deconvolving the measured diffusion MR signal with the response function (Tournier *et al.*, 2004). This deconvolution process is susceptible to noise contamination, however, which can lead to the introduction of artefactual (physically impossible) negative regions in the FOD. "Constrained" spherical deconvolution addresses this limitation of spherical deconvolution by imposing a constraint on such negative regions.

This approach provides FOD estimates that are robust to noise whilst preserving angular resolution (Tournier *et al.*, 2007). The CSD technique is computationally simple and very fast, the diffusion data for CSD can be acquired with clinically feasible settings, no a priori information is needed regarding the number of distinct fibre orientations and fibre orientations are estimated directly - it is not dependant on any assumed model of diffusion (Tournier *et al.*, 2004). Importantly, this approach overcomes partial volume effects associated with diffusion tensor imaging, allows reliable estimation of diffusion measures and permits fibre-tracking through regions of crossing fibres (Tournier *et al.*, 2008).

1.4. Inter- and intra-hemispheric tracts isolated in this study

To look for evidence of reduced structural integrity of white matter in key inter-hemispheric and intra-hemispheric tracts in autism, two major intra-hemispheric association tracts [inferior fronto-occipital fasciculus (IFOF) and arcuate fasciculus (AF)] and two inter-hemispheric callosal tracts (forceps major and forceps minor) were examined using a CSD-based tractography approach in this study. These white matter tracts were selected for a number of reasons. (1) The IFOF and the AF link frontal and posterior brain regions that have repeatedly shown abnormal functional connectivity in autism, e.g. (Just *et al.*, 2004; Just *et al.*, 2007). White matter organization in these tracts has not been well studied in autism. Knowledge of white matter micro- and macrostructure in these fronto-posterior tracts is crucial to understanding the anatomical correlates of abnormal fronto-posterior functional connectivity in autism. (2) The AF in the right hemisphere is known to be involved in visuospatial processing (Doricchi *et al.*, 2008; Thiebaut de Schotten *et al.*, 2008) and the IFOF is also thought to play a role in visual processing (Catani and Thiebaut de Schotten, 2008; Fox *et al.*, 2008; Rudrauf *et al.*, 2008). The functional role of these tracts in visuospatial processing is of particular relevance to this study as behavioural, functional MRI and functional connectivity MRI data from a visuospatial processing task were available on 42 of the 50 study participants who completed the DTI scan for this study, thus allowing for an investigation of relationships between brain structure (diffusion measures) and brain function. (3) The

IFOF, AF, forceps major and forceps minor were also selected for examination in this study as they were implicated by the functional connectivity MRI (fcMRI) data (discussed in Chapter 3) and TBSS data (discussed in Chapter 4). The fcMRI data was overlaid on the white matter skeleton in FSL (http://www.fmrib.ox.ac.uk/fsl/) and the Johns Hopkins University DTI-based WM atlas was used to determine which white matter tracts either ran through or were in close proximity to the fcMRI defined regions of interest. Brain regions that showed abnormal functional connectivity in autism during visuospatial processing were in close proximity to the IFOF, AF and genu and splenium of the corpus callosum.

1.5. Aim and hypothesis

The main aim of this study was to isolate and characterize major inter- and intra-hemispheric white matter tracts in autism using CSD-based fibre tractography in order to investigate white matter organization in specific tracts that are important in visuospatial processing. CSD-based tractography avoids a number of critical confounds that are associated with diffusion tensor tractography, and to our knowledge, this is the first time that this advanced diffusion tractography method has been used in autism research. Based on the limited literature, we hypothesized that there would be significant disruption of white matter microstructure in individuals with ASD. It was hoped that investigation of white matter in specific tracts involved in visuospatial processing would shed light on the underlying pathophysiology of the behavioural and functional differences that were observed during mental rotation in ASD.

2. Methods

2.1. Participants, data acquisition and data pre-processing

Participants for this study, data acquisition and pre-processing of the diffusion weighted-MRI data are outlined in the Methods section of chapter 4 (sections 2.1 - 2.3). In summary twenty-five participants with ASD and twenty-five matched controls completed a High Angular Resolution Diffusion Imaging (HARDI) scan, which was pre-processed using an optimised

protocol within the diffusion MR toolbox ExploreDTI (http://www.ExploreDTI.com) (Leemans *et al.*, 2009).

2.2. Tractography Approach

Constrained spherical deconvolution (CSD) was used to extract the fibre orientation distribution (FOD) from the diffusion weighted signal in each voxel (Tournier *et al.*, 2007). The IFOF, AF, forceps major and forceps minor white matter tracts were reconstructed based on this FOD (Jeurissen *et al.*, 2011). The fibre-tracking algorithm is based on the estimated FOD obtained from CSD and involves the following steps: 1. Fibre tracking starts at a given seed point where the diffusion-weighted signal at the current position of the trajectory is obtained using trilinear interpolation, 2. CSD is used to estimate the FOD, 3. The FOD peak direction that was closest to the previous stepping direction is extracted, 4. The trajectory is advanced by a fixed step size (1mm) along the obtained direction. Tracking ends when the FOD peak intensities are beneath a fixed threshold (i.e. 0.1), a maximum angle (30°) is exceeded, or the tract leaves the specified brain mask (Jeurissen *et al.*, 2011).

A multiple ROI approach was employed to isolate the fibres in the tracts of interest. This approach has been shown to ensure robust recovery of the major fibre tracts in the human brain (Mori *et al.*, 2002; Wakana *et al.*, 2007). To ensure consistency in extracting the tracts across subjects, all tracts of interest were extracted and quantified in native space using the protocols outlined below.

2.3. Fibre extraction protocol for each tract of interest

2.3.1. Inferior fronto-occipital fasciculus

The IFOF is a long white matter association tract that links the occipital lobe and the orbitofrontal cortex. Two "AND" regions of interest (ROI) - the term "AND" indicates that fibres must pass through this ROI - were defined to dissect the fibres of the left IFOF and the right IFOF (see Figure 1). ROI_1 was drawn around the occipital lobe on a coronal slice at the mid-point between the posterior edge of the cingulum and the posterior edge of the parieto-occipital sulcus. The second ROI was drawn around the IFOF

(identified as an intense green circular tract in Figure 1) on a coronal slice in the frontal lobe at the location where frontal and temporal lobes are separated.

2.3.2. Arcuate fasciculus

The arcuate fasciculus is a white matter association tract that is composed of long and short fibres linking the perisylvian cortex of frontal, parietal and temporal lobes. It comprises the temporal part of the superior longitudinal fasciculus (SLF). Two "AND" ROIs were defined to isolate the left and the right arcuate fasciculus (Wakana *et al.*, 2007) (see Figure 2). ROI_1 was drawn on a coronal slice at the level of the middle of the posterior limb of the internal capsule. This ROI was drawn around the core of the SLF (identified as an intense green tract with a triangular shape in Figure 2) and all branches coming out from this core. ROI_2 was drawn on an axial slice at the level of the inferior part of the splenium around projections lateral to the sagittal stratum.

2.3.3. Forceps major

The forceps major is a callosal interhemispheric white matter tract that links the bilateral occipital lobes through the splenium. Four ROIs were used to isolate the forceps major (see Figure 3). As described by Wakana et al (2007) a coronal plane was selected at the most posterior part of the parieto-occipital sulcus and "AND" gates were drawn in each hemisphere around the occipital lobe (ROI_1 and ROI_2) (Wakana *et al.*, 2007). As there is substantial individual variability in the morphology of the corpus callosum, a third "AND" ROI (ROI_3) was drawn around the splenium of the corpus callosum, which was delineated on the mid-sagittal plane as the bulbous portion of the callosum that was inferior to the posterior aspect of the callosum (Thomas *et al.*, 2009). ROI_4 was a "NOT" ROI – the term "NOT" indicates that any fibres passing through this ROI are excluded . ROI_4 was drawn on the mid-sagittal slice posterior to the splenium of the corpus callosum to ensure the exclusion of spurious interhemispheric non-forceps major tracts.

2.3.4. Forceps minor

The forceps minor is a callosal interhemispheric white matter tract that links the bilateral frontal lobes through the rostrum and genu. Four ROIs were used to isolate the forceps minor (see Figure 4). On the mid-sagittal plane the mid-point between the anterior tip of the frontal lobe and the anterior edge of the genu of the corpus callosum was identified. The coronal slice at this level was selected and "AND" gates were drawn in both hemispheres around the entire frontal lobe (ROI_1 and ROI_2). As for the forceps major, a third "AND" ROI (ROI_3) was drawn around the genu of the corpus callosum, which was delineated on the mid-sagittal plane as the region of the corpus callosum anterior to the body, to control for individual variability in callosum morphometry (Thomas *et al.*, 2009). A "NOT" gate was drawn on the mid-sagittal plane anterior to the genu of the corpus callosum to ensure that spurious non forceps minor tracts were excluded (ROI_4).

2.4. Dependant measures

For each tract in each participant, microstructural measures of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), the Westin measures of linear diffusion co-efficient (CL) and planar diffusion co-efficient (CP) (Westin *et al.*, 2002) and a macrostructural measure of tract volume was computed from the tracts (Lebel *et al.*, 2008). These diffusion metrics are discussed in detail in chapter 1, section 2.2.2.

2.5. Statistical analysis

Statistical comparisons of the data were performed using PASW (SPSS) software version 18 (SPSS Inc., Chicago, IL). For all analyses the level of statistical significance was defined as $p < 0.05$ (two-tailed) and Bonferroni corrections were used throughout the analyses. For the IFOF and the AF, univariate ANOVA with Group (ASD / Control) as the between-subjects factor was performed for the dependant measures FA, MD, RD, AD, CP, CL and tract volume. In addition, a "lateralisation index" was calculated using the formula [(Left – Right) / (Left + Right)] for each dependant measure for the IFOF and AF tracts. The purpose of this lateralisation index was to derive a summary statistic that quantified the degree of hemispheric asymmetry for each participant. The index values range from +1 to -1 with a

positive value indicating greater left hemisphere asymmetry (Thomas *et al.*, 2011). A univariate ANOVA was subsequently performed to investigate group differences in this lateralisation index. As hemispheric asymmetry is not relevant for the two interhemispheric tracts, forceps major and forceps minor, a univariate ANOVA was performed to investigate between-group differences in each of the dependant measures.

To specifically explore an association between white matter integrity and behavioural performance during visuospatial processing, bivariate Pearson correlations were used to examine relationships between diffusion and lateralisation measures that showed significant abnormality in the ASD group, and mean response times during a visuospatial processing task. Details of the mental rotation paradigm are outlined in chapter 2, section 2.2. Pearson correlation analysis was used as mean response times in this study were normally distributed, as indicated by p values of >0.05 following Kolmgorov-Smirnov and Shapiro-Wilk tests of normality. To investigate how white matter integrity may influence behaviour, an exploratory correlation analysis also investigated the relationships between autism severity (as measured by ADOS scores for communication/social interaction and stereotyped behaviours/restricted interests) and diffusion and lateralisation measures that showed significant abnormality in the ASD group using bivariate Spearman's correlation analysis. Spearman's correlation analysis was used as ADOS scores are not normally distributed.

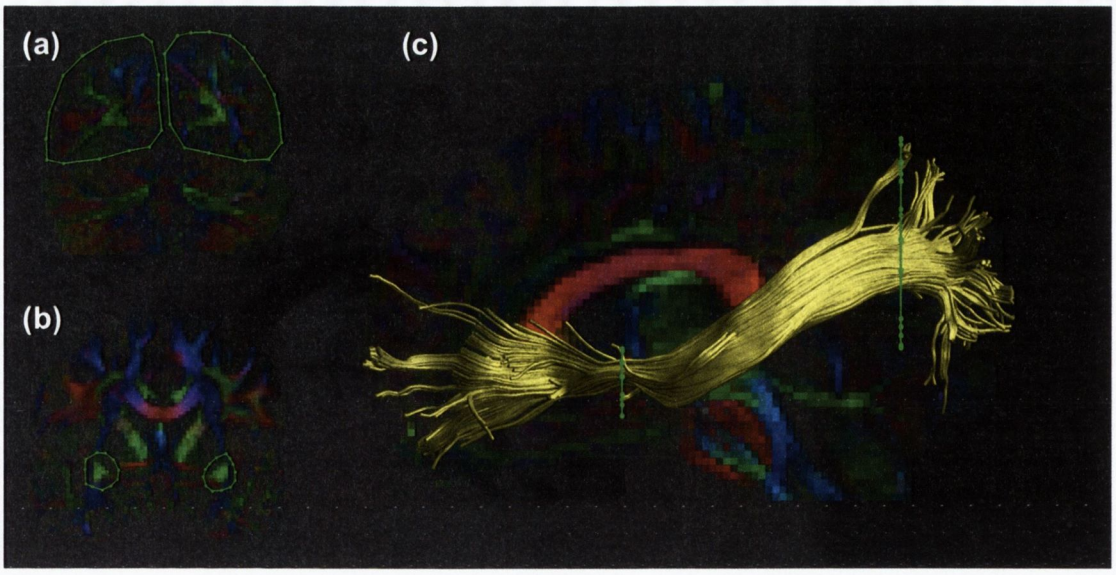


Figure 1: Illustration of the protocol used for extraction of the IFOF. (a) Green lines demarcate "AND" ROI_1 for the left and right IFOF, (b) "AND" ROI_2 for the left and right IFOF, (c) Sagittal view of left IFOF and ROI_1 and ROI_2; green vertical lines show the positions of ROI_1 and ROI_2 in the sagittal plane.

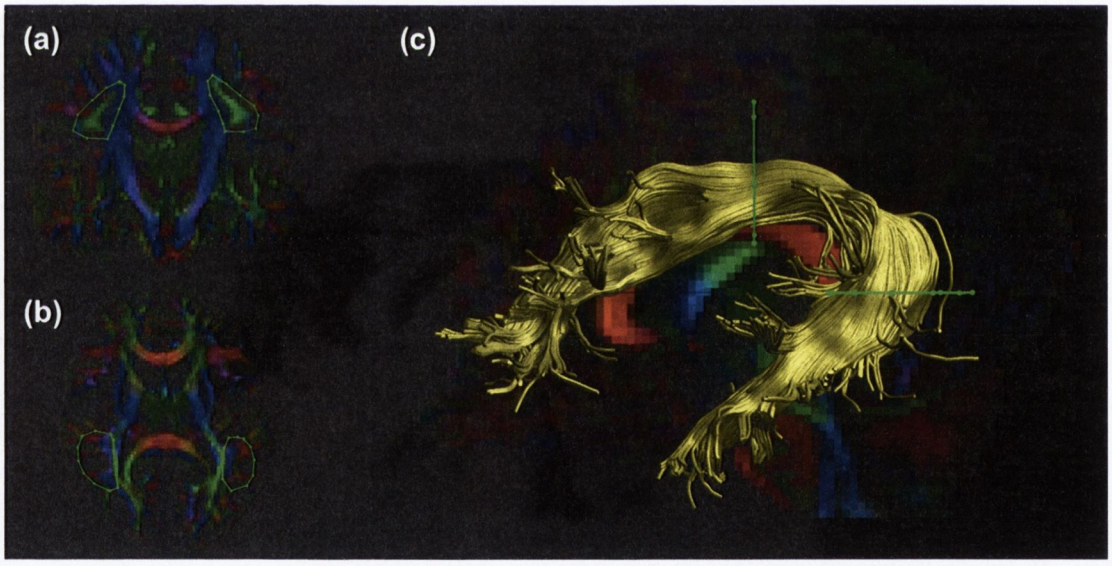


Figure 2: Illustration of the protocol used for extraction of the AF. (a) Green lines demarcate "AND" ROI_1 for the left and right AF on a coronal slice, (b) "AND" ROI_2 for the left and right AF on an axial slice, (c) Sagittal view of left AF and ROI_1 and ROI_2; green vertical and horizonal line show positions of ROI_1 and ROI_2 in the sagittal plane.

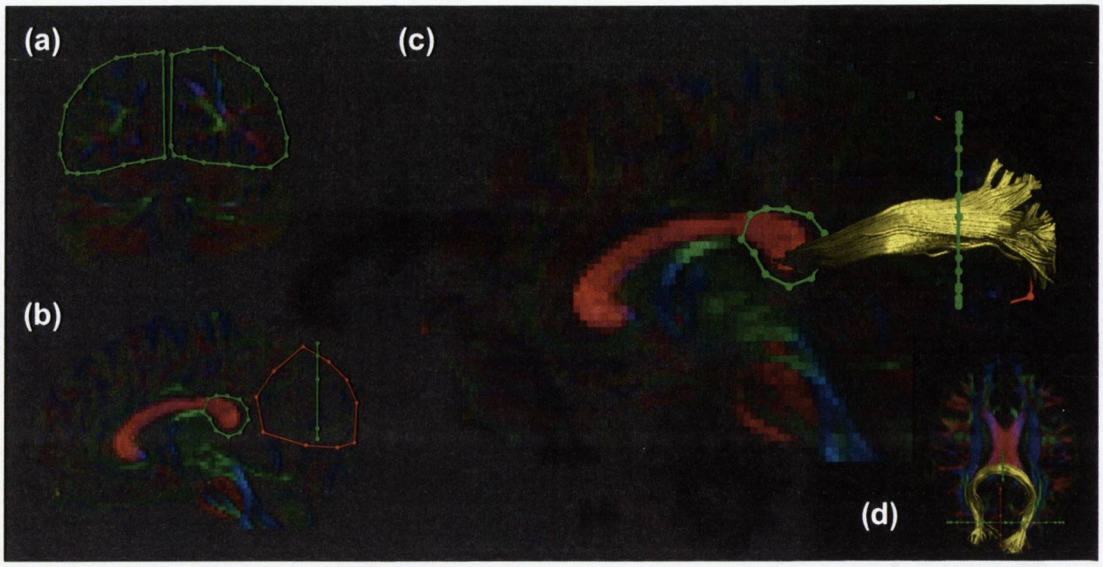


Figure 3: Illustration of the protocol used for extraction of the Forceps major. (a)"AND" ROI_1 and ROI_2 for the Forceps major, (b) "AND" ROI_3 (green circle) delineates the splenium of the corpus callosum, "NOT" ROI_4 (red) (c) Sagittal view of Forceps major; green vertical line shows the positions of ROI_1 and ROI_2 in the sagittal plane (d) axial view of forceps major.

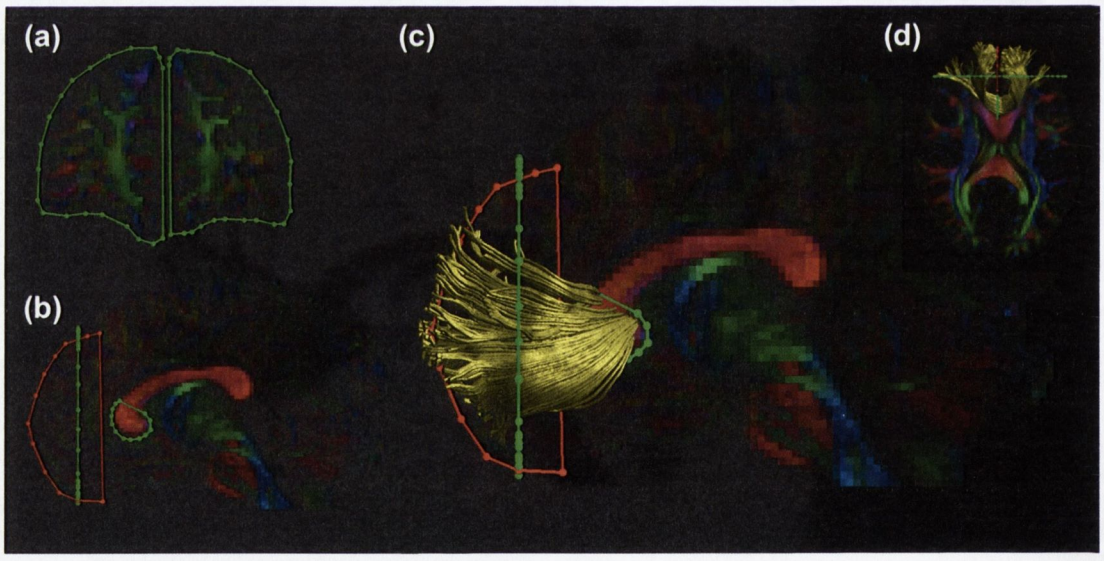


Figure 4: Illustration of the protocol used for extraction of the Forceps minor. (a)"AND" ROI_1 and ROI_2 for the Forceps minor, (b) "AND" ROI_3 (green circle) delineates the genu of the corpus callosum, "NOT" ROI_4 (red line), (c) sagittal view of Forceps minor; green vertical line shows the positions of ROI_1 and ROI_2 in the sagittal plane, (d) axial view of forceps minor.

3. Results

3.1. Between-group differences in dependant measures

In the right IFOF, mean FA was significantly lower in the ASD group (ASD mean FA 0.47, control mean FA 0.48, $F(1,48) = 5.143$, $p<0.028$, $\eta p2=0.097$), mean CL was significantly lower in the ASD group (ASD mean CL 0.44, control mean CL 0.46, $F(1,48) = 6.724$, $p<0.013$, $\eta p2=0.123$) and mean CP was significantly higher in the ASD group (ASD mean CP 0.17, control mean CP 0.16, $F(1,48) = 5.25$, $p<0.026$, $\eta p2=0.099$). Mean AD was lower in the ASD group relative to controls in this tract; however this difference only approached statistical significance (mean AD ASD 0.0011, mean AD controls 0.0012, $F(1, 48) = 3.95$, $p < 0.053$, $\eta p2=0.076$). There were no between-group differences in ADC, RD and volume (all $p>0.05$).

In the left IFOF, in bilateral arcuate fasciculus and in the forceps minor, there were no between-group differences in micro- and macrostructural diffusion measures (see Table 1).

In the forceps major, there was significantly reduced CL in the ASD group relative to controls (ASD mean CL 0.568, control mean CL 0.584, $F(1,48) = 5.449$, $p<0.024$, $\eta p2=0.102$). Of note, mean FA in the forceps major was lower in the ASD group than in the control group (mean FA ASD 0.57, mean FA controls 0.59) and this difference showed a trend towards significance ($p<0.091$). This trend was examined more closely by examining the interquartile ranges of FA, which showed that FA in the lower interquartile range (IQR_L) was significantly lower in the ASD group (IQR_L FA ASD 0.42, IQR_L FA controls 0.44, $p<0.040$). This finding may be suggestive of a subtle increase in white matter disruption in autism that is not captured by the more general comparison of mean FA.

		Right Hemisphere				Left Hemisphere				Interhemispheric			
Measure	**Group**	**AF Mean (SD)**	**p**	**IFOF Mean (SD)**	**p**	**AF Mean (SD)**	**p**	**IFOF Mean (SD)**	**p**	**F. major Mean (SD)**	**p**	**F. minor Mean (SD)**	**p**
FA	**ASD**	0.40 (0.02)	0.76	0.47* (0.02)	**0.02***	0.42 (0.02)	0.85	0.47 (0.02)	0.26	0.57 (0.03)	0.09	0.51 (0.02)	0.79
	Con	0.39 (0.01)		0.48* (0.02)		0.43 (0.02)		0.48 (0.02)		0.59 (0.03)		0.51 (0.02)	
ADC (mm^2/sec)	**ASD**	0.000693 (0.00002)	0.14	0.000756 (0.00002)	0.99	0.000696 (0.00002)	0.14	0.000758 (0.00002)	0.25	0.000866 (0.00005)	0.42	0.000753 (0.00002)	0.73
	Con	0.000686 (0.00002)		0.000756 (0.00002)		0.000689 (0.00001)		0.000753 (0.00002)		0.000855 (0.00005)		0.000752 (0.00002)	
AD (mm^2/sec)	**ASD**	0.00101 (0.00002)	0.12	0.00119 (0.00003)	0.053	0.00104 (0.00002)	0.23	0.00120 (0.00004)	0.92	0.00151 (0.00006)	0.81	0.00126 (0.00004)	0.63
	Con	0.00100 (0.00003)		0.00121 (0.00004)		0.00103 (0.00003)		0.00120 (0.00003)		0.00152 (0.00006)		0.00125 (0.00003)	
RD (mm^2/sec)	**ASD**	0.000533 (0.00002)	0.36	0.000540 (0.00002)	0.11	0.000525 (0.00002)	0.28	0.000540 (0.00002)	0.21	0.000542 (0.00005)	0.20	0.000502 (0.00002)	0.91
	Con	0.000528 (0.00002)		0.000530 (0.00002)		0.000520 (0.00002)		0.000530 (0.00002)		0.000523 (0.00005)		0.000501 (0.00002)	
CP	**ASD**	0.186 (0.017)	0.32	0.170* (0.013)	**0.01***	0.210 (0.015)	0.46	0.162 (0.013)	0.49	0.108 (0.011)	0.07	0.144 (0.010)	0.22
	Con	0.190 (0.015)		0.161* (0.014)		0.213 (0.015)		0.165 (0.016)		0.102 (0.012)		0.147 (0.008)	
CL	**ASD**	0.361 (0.025)	0.47	0.444* (0.023)	**0.03***	0.371 (0.020)	0.75	0.455 (0.025)	0.49	0.568* (0.025)	**0.02***	0.489 (0.021)	0.98
	Con	0.357 (0.016)		0.463* (0.027)		0.369 (0.022)		0.460 (0.030)		0.584* (0.022)		0.489 (0.017)	
Volume (mm^3)	**ASD**	23839 (5923)	0.61	18347 (4824)	0.23	27679 (6537)	0.65	19889 (4890)	0.41	18060 (5263)	0.97	35964 (8150)	0.89
	Con	24693 (5897)		20090 (5373)		28475 (6053)		18640 (5836)		18112 (4862)		35631 (9367)	

Table 1: Caption on following page

Caption for Table 1: The mean, standard deviation (SD) and p values for the six microstructural dependant measures (FA, AD, RD, ADC, CP, CL) and the macrostructural measure (tract volume) for the arcuate fasciculus (AF), inferior fronto-occipital fasciculus (IFOF), Forceps major (F. major) and Forceps minor (F. minor) in the ASD and control groups. * indicates significant between-group differences with $p<0.05$.

3.2. Lateralisation Index analysis

In the IFOF, relative to controls, the ASD group showed significantly greater left lateralization of ADC (ASD 0.0012, Control -0.0025 $F(1, 48) = 4.07$, $p < 0.049$, $\eta p2=0.078$), AD (ASD 0.0046, Control -0.0039 $F(1, 48) = 6.59$, $p < 0.013$, $\eta p2=0.121$) and CL (ASD 0.012, Control -0.0031 $F(1, 48) = 4.35$, $p < 0.042$, $\eta p2=0.083$) and significantly reduced left lateralisation of CP (ASD -0.025, Control 0.010 $F(1, 48) = 5.74$, $p < 0.021$, $\eta p2=0.107$). Increased left lateralisation of volume in the ASD group approached significance ($p<0.051$).There was no between-group difference in lateralisation of RD ($p>0.05$) (see Table 2).

In the AF, there were no between-group differences in lateralisation of FA, ADC, AD, RD, CP, CL or volume (see Table 2).

Measure	Group	AF mean (SD)	p value	IFOF mean (SD)	p value
FA	ASD	0.0328 (0.021)	0.70	0.0058 (0.020)	0.19
	Con	0.0353 (0.025)		-0.0012 (0.017)	
ADC	ASD	0.0014 (0.005)	0.78	0.0012 (0.006)	0.04*
	Con	0.0018 (0.004)		-0.0025 (0.006)	
AD	ASD	0.0108 (0.009)	0.55	0.0046 (0.011)	0.01*
	Con	0.0125 (0.010)		-0.0039 (0.012)	
RD	ASD	-0.0077 (0.009)	0.73	-0.0026 (0.013)	0.61
	Con	-0.0088 (0.012)		-0.0008 (0.011)	
CP	ASD	0.0612 (0.051)	0.71	-0.0250 (0.048)	0.02*
	Con	0.0560 (0.040)		0.0100 (0.054)	
CL	ASD	0.0140 (0.031)	0.74	0.0120 (0.026)	0.04*
	Con	0.0170 (0.028)		-0.0031 (0.024)	
Volume	ASD	0.0760 (0.099)	0.95	0.0420 (0.151)	0.051
	Con	0.0740 (0.099)		-0.0470 (0.160)	

Table 2: Lateralisation indices for the six micro-structural dependant measures (FA, AD, RD, ADC, CP, CL) and the macro-structural measure (tract volume) for the AF and IFOF. * indicates significant between-group differences with $p<0.05$.

3.3. Correlation analysis

To investigate the behavioural ramifications of the disrupted white matter integrity in the ASD group, correlation analysis was used to examine the relationships between visuospatial processing performance, autism severity, and diffusion and lateralisation measures that showed significant abnormality in the ASD group. Therefore diffusion measures used in correlation analyses included mean FA, mean CP and mean CL in right IFOF and mean FA and mean CL in Forceps major. The lateralisation measures used in correlation analyses included the lateralisation indices for ASD and control groups for ADC, AD, CL and CP for the IFOF. The lateralisation index for volume was also included in these exploratory correlation analyses as the between-group difference approached significance ($p<0.051$).

3.3.1. Correlation analysis of mean response time during mental rotation

Correlation analysis revealed a significant negative correlation between mean response time during the mental rotation task and mean FA in the right IFOF (Pearson correlation (r) -0.457, $p<0.037$) in the ASD group. There was no correlation between these variables in the control group. The correlation analyses for mean response time and CL in right IFOF, FA in forceps major and CL in forceps major all showed a trend towards statistical significance in the ASD group ($p<0.060$, $p<0.063$, $p<0.088$ respectively) whereas the control group showed no such trend ($p<0.389$, $p<0.918$, $p<0.871$) (see Table 3).

In the ASD group but not in controls, there was a significant positive correlation between the lateralisation index of CL and mean response time (r 0.436, $p<0.048$). This correlation suggests an association between increasing left lateralisation of CL and slower mean response times (equating to poorer visuospatial processing) in the ASD group. An interpretation of the functional significance of these correlations is offered in the Discussion.

3.3.2. Correlation analysis of ADOS scores

Correlation analysis revealed a significant positive correlation between the ADOS score for communication and social interaction and the lateralisation index of volume (r_s 0.472, $p<0.017$). This correlation suggests an association between greater left lateralisation of volume of the IFOF and increased severity of communication and reciprocal social interaction impairments in the ASD group. There were no correlations between the ADOS score for stereotyped behaviours and restricted interests and microstructural diffusion measures or lateralisation indices (see Table 4).

	Behavioural measure Mean Response Time		
Diffusion measure	**Group**	**r**	**p**
FA Right IFOF	ASD	-0.457*	0.037*
	Control	0.238	0.298
CP Right IFOF	ASD	0.150	0.517
	Control	-0.074	0.749
CL Right IFOF	ASD	-0.417	0.060
	Control	0.198	0.389
FA F. major	ASD	-0.412	0.063
	Control	-0.024	0.918
CL F. major	ASD	-0.381	0.088
	Control	0.038	0.871
LI ADC	ASD	-0.264	0.248
	Control	0.275	0.228
LI AD	ASD	0.202	0.381
	Control	-0.104	0.654
LI CL	ASD	0.436*	0.048*
	Control	-0.290	0.202
LI CP	ASD	-0.181	0.434
	Control	0.130	0.574
LI_Volume	ASD	-0.383	0.087
	Control	0.162	0.483

Table 3: Results of bivariate Pearson correlation analyses between mean response time (MRT) during visuospatial processing and all diffusion and lateralisation measures showing abnormality in the ASD group. LI: Lateralisation index, r: Pearson correlation co-efficient. *Indicates statistical significance ($p<0.05$).

	Behavioural measure	
	ADOS C/SI	ADOS RB/SI
Diffusion measure	**r_s (p)**	**r_s (p)**
FA Right IFOF	0.006 (0.979)	0.025 (0.907)
CP Right IFOF	0.241 (0.247)	0.141 (0.501)
CL Right IFOF	-0.116 (0.581)	-0.068 (0.745)
FA Forceps major	0.188 (0.368)	-0.350 (0.086)
CL Forceps major	0.220 (0.292)	-0.282 (0.173)
LI ADC	-0.269 (0.193)	-0.123 (0.559)
LI AD	0.060 (0.775)	0.101 (0.632)
LI CL	0.176 (0.399)	0.175 (0.403)
LI CP	0.007 (0.972)	-0.253 (0.223)
LI_Volume	0.472 (0.017)*	0.169 (0.418)

Table 4: Results of bivariate Spearman's correlation analyses between ADOS scores and all diffusion and lateralisation measures showing abnormality in the ASD group. (LI: Lateralisation index, r_s: Spearman's correlation co-efficient, ADOS C/SI: ADOS score for Communication and Reciprocal Social Interaction, ADOS SB/RI: ADOS score for Stereotyped Behaviours and Restricted Interests, *indicates statistical significance ($p<0.05$)).

4. Discussion

The major finding from this study is that there is disruption of white matter in inter and intra-hemispheric white matter tracts in ASD that is associated with both visuospatial processing performance and autism severity. Significant alteration of white matter organization was present in the right IFOF and the forceps major. Poorer visuospatial processing was associated with disrupted white matter in the right IFOF and there was a relationship between increased severity of ASD (as measured by ADOS score for communication and reciprocal social interaction) and increased left lateralization of volume in the IFOF.

4.1. Inferior fronto-occipital fasciculus

The IFOF is a major long-range white matter association tract connecting ipsilateral frontal and occipital lobes and ipsilateral frontal and posterior parietal and temporal lobes (Martino *et al.*, 2010). The function of the IFOF is poorly understood but it is thought to play an important role in visual processing, reading, attention, the semantic system and, of particular relevance to autism, in correctly recognizing emotion in faces (Catani and Mesulam, 2008; Doricchi *et al.*, 2008; Epelbaum *et al.*, 2008; Fox *et al.*, 2008; Rudrauf *et al.*, 2008; Philippi *et al.*, 2009; Martino *et al.*, 2010). A finding of reduced structural connectivity in this extensive fronto-posterior tract would be in keeping with the theory of long-range fronto-posterior underconnectivity in autism. It is perhaps surprising therefore that only three tractography studies have examined white matter integrity of the IFOF in autism. These three studies used diffusion tensor tractography, an approach with significant limitations that were outlined in the introduction to this chapter. One of these studies reported increased left lateralization of volume in the IFOF in adults with ASD (Thomas *et al.*, 2011) but the two other studies found no abnormalities in this tract in individuals with autism (Pugliese *et al.*, 2009; Kumar *et al.*, 2010).

Significant microstructural abnormalities of the right IFOF were present in the ASD group in this study. Relative to controls, participants with ASD had reduced FA, reduced CL and increased CP in this tract. CL reflects the

uniformity of tract direction within a voxel and a measure of high CL in a voxel implies that there is only one dominant fibre orientation (Westin *et al.*, 2002; Vos *et al.*, 2012). A measure of high CP implies the presence of complex fibre architecture, i.e. the presence of more than one dominant fibre orientation within the voxel (Vos *et al.*, 2012). The findings of reduced FA and CL and increased CP in the IFOF suggest that there is a greater level of complex fibre architecture in the right IFOF in the ASD group in this study. Interestingly, it has been reported in a large study of patients with focal brain lesions that damage associated with the right IFOF was associated with significant deficits in facial emotion recognition with specific deficits recognizing sadness, anger and fear (Philippi *et al.*, 2009). Impairment of emotion recognition is extensively documented in autism and the finding of microstructural abnormalities in the right IFOF in ASD is exciting as it offers an insight into white matter abnormalities that may underpin a core deficit in the condition.

Correlation analysis revealed an intriguing relationship between white matter integrity in the right IFOF and behavioural performance during a visuospatial processing task. In the ASD group (but not controls), there was a negative correlation between FA in the right IFOF and mean response times during mental rotation indicating an association between reduced white matter integrity and poorer visuospatial processing. Interestingly, there was also a strong trend ($p<0.060$) towards a similar negative correlation between mean CL in the right IFOF and mean response times during mental rotation in the ASD group, but not in controls. Taken together, these findings suggest that as the level of white matter organization in the right IFOF diminishes, there is a detrimental effect on visuospatial processing speed in ASD. These correlations are particularly interesting as they reveal an association between atypical visuospatial function in autism and specific structural brain abnormalities. During mental rotation, the participants with ASD in this study showed significant differences in behavioural performance, brain activation and functional connectivity compared with controls (discussed in chapters 2 and 3). The pathophysiology of these functional and behavioural differences is unknown, but these correlations provide an intruiging insight into possible

aetiological structural abnormalities. A small number of studies have previously described correlations that suggest that there are indeed functional consequences of disrupted white matter in autism (Cherkassky *et al.*, 2006; Alexander *et al.*, 2007a; Just *et al.*, 2007; Mason *et al.*, 2008; Thakkar *et al.*, 2008; Damarla *et al.*, 2010; Sahyoun *et al.*, 2010b).

A test of the lateralization of DTI measurements in the IFOF showed that there was significantly greater right lateralization of CP and greater left lateralization of AD, ADC and CL in the ASD group relative to controls. There was also a strong trend towards greater left lateralization of volume in the ASD group ($p<0.051$). The right lateralization of CP emerged from a right-sided increase in CP in the ASD group. In contrast, the left lateralization of AD, ADC and CL appeared to be driven by the right-sided reduction in these measures in the ASD group relative to controls. With regard to lateralization of volume, controls showed a reduction in left relative to right IFOF volume whereas conversely, the ASD group showed an increase in volume of left relative to right IFOF. No previous study has examined lateralization patterns specifically in the IFOF in autism, but one previous study investigating intrahemispheric lateralization differences in autism reported greater left lateralization of number of streamlines and number of voxels in three intrahemispheric tracts combined together (IFOF, ILF and UF) in ASD (Thomas *et al.*, 2011). In relation to visual perception, it is known that the right hemisphere is more sensitive to global information whereas the left hemisphere is more sensitive to information about local features (Robertson and Ivry, 2000; Yovel *et al.*, 2001). Thomas et al (2011) speculated that the increased left lateralization of volume in ASD might be the source of the local visual bias that has been reported in individuals with autism (Happe and Frith, 2006), but they did not find any correlation between increased left lateralization of volume and autism severity (measured by ADI diagnostic scores). In this study, there was a positive correlation between left lateralization of volume and the ADOS score for communication and reciprocal social interaction. This is interesting as it provides preliminary evidence to suggest that increased left lateralization of volume of the right IFOF is related to autism symptomatology. Increased left

lateralization of CL was also correlated positively with visuospatial processing speed. At first, this correlation appears to suggest that left lateralization of CL is detrimental to visuospatial processing. The increased left lateralization of CL in this study, however, is driven by the reduction in CL in the right IFOF, not by increased structural connectivity (increased CL) in the left hemisphere IFOF. It is possible therefore that the local visual bias in autism does not arise from increased structural connectivity within the left hemisphere, but instead results from a compensatory increased use of the unimpaired left hemisphere in the face of right hemisphere white matter dysfunction.

4.2. Arcuate fasciculus

The arcuate fasciculus (AF) connects the perisylvian cortex of the frontal, parietal and temporal lobes. In the right hemisphere this tract has a function in visuospatial processing (Doricchi *et al.*, 2008; Thiebaut de Schotten *et al.*, 2008) and the left hemisphere AF has a well-established role in language and praxis. White matter microstructure of the right and left AF did not differ between groups in this study, nor were there between-group differences in lateralization. Previous tractography studies investigating the AF in individuals with ASD have shown reduced FA, increased MD and reduced left lateralization of FA, MD, volume and average fibre length (Fletcher *et al.*, 2010; Kumar *et al.*, 2010; Lo *et al.*, 2011; Wan *et al.*, 2012). One possible reason for the lack of between-group differences in white matter organization in the AF is the methodology that was used in this study. CSD based tractography allows full isolation of the frontal projections of the AF. In previous studies it is possible that where the AF passes through regions of crossing fibres in the frontal lobe, fibre tracking terminated prematurely. This would lead to extraction of diffusion measurements from only a segment of the AF rather than the whole tract. It is also important to note that our study population was verbal, with average or above average IQ. It is possible that individuals with ASD but normal language function do not have significant disruption of white matter in this tract (although see (Kumar *et al.*, 2010; Lo *et al.*, 2011).

4.3. Forceps major

The forceps major links left and right occipital lobes through the splenium and appears to play an important role in visual perception, manipulation of visuospatial information, memory and topographical orientation (Rudge and Warrington, 1991; Tamura *et al.*, 2007). Previous tractography studies that have examined the forceps major have shown mixed results with one study showing increased ADC (Hong *et al.*, 2011) but three others failing to find abnormalities in this tract (Thomas *et al.*, 2011; Weinstein *et al.*, 2011; Wolff *et al.*, 2012). In this study there was significant disruption of white matter of the forceps major in ASD. One possible reason for the varying results from these studies is that the participants in these studies are in different age ranges. The study population that most closely resembles this study in terms of age is that of Hong et al (2011) who did report reduced integrity of white matter in the forceps major. Of the studies that did not find abnormalities, two were carried out in very young infants with autism, the other in adults with ASD.

4.4. Forceps minor

The forceps minor connects the bilateral prefrontal cortex through the genu and rostrum of the corpus callosum. No micro- or macrostructural abnormalities of this tract were present in this study. Only two other studies have specifically investigated white matter of the forceps minor in autism. Increased FA in this tract was reported in 6 month old infants who subsequently developed autism, but this difference in FA had disappeared when these infants were 12 and 24 months old (Wolff *et al.*, 2012). In adults with ASD, macrostructural abnormalities of the forceps minor were present, including reduced number of streamlines and reduced number of voxels in ASD, but there were no microstructural abnormalities of white matter in this tract (Thomas *et al.*, 2011).

4.5. Study limitations

As mentioned in previous chapters, participants with ASD were limited to male, right-handed individuals with average or above average IQ. Therefore results are very specific to this group and are not representative for all individuals on the spectrum. In addition, this study selected only two intra-

hemispheric tracts and two inter-hemispheric tracts, however it is possible that there are white matter abnormalities in a number of other white matter tracts in autism that were not investigated here. Finally, although ADOS scores are widely used as a measure of autism severity, these data are ordinal and it would be preferable to use a quantitative measure of ASD severity in cases and controls, for example the Social Responsiveness Scale (SRS) (Constantino *et al.*, 2003).

5. Conclusion

Using a novel and advanced tractography method to isolate major inter- and intra-hemispheric white matter tracts in autism, this study has demonstrated that there are significant alterations in the microstructural organization of white matter in the right IFOF and forceps major in ASD, which are correlated with visuospatial processing performance and with autism severity. In the previous chapters, it was shown that during mental rotation, participants with ASD in this study showed significant differences in behavioural performance, brain activation and functional connectivity compared with controls. The pathophysiology of these functional and behavioural differences is unknown but the association between reduced white matter integrity in the right IFOF and poorer visuospatial processing performance revealed in this study provides an insight into structural brain abnormalities that may influence atypical visuospatial processing in autism.

6. Investigating the links between structural connectivity, functional connectivity and behaviour in ASD

1. Introduction

There is extensive evidence to suggest that autism is a disorder characterized by disrupted functional and structural neural connectivity. Abnormal inter- and intra-regional functional connectivity has been described whilst participants have performed various neuropsychological paradigms and whilst in the resting state. In parallel with functional connectivity research, a number of diffusion imaging studies in autism have demonstrated aberrant 'structural connectivity' - a term referring to the integrity of white matter micro- and macrostructure. Abnormal functional connectivity between brain regions in autism may arise from disrupted organization of white matter, but its pathophysiology is unknown. Despite the numerous studies that have consistently reported abnormal functional connectivity in autism, there is a surprising lack of research attempting to integrate analysis of functional and structural connectivity in the same study population, an approach that may provide key insights into the specific neurobiological underpinnings of altered functional connectivity in autism.

1.1. Behavioural consequences of disrupted neural connectivity in ASD

There appear to be significant behavioural consequences of disrupted structural and functional connectivity in autism. In relation to structural connectivity, several studies have explored the relationship between white matter organization and autism symptom severity. Increased severity of restricted and repetitive behaviours was correlated with reduced FA in left precentral gyrus and posterior brain regions (Cheung *et al.*, 2009), right anterior cingulate cortex (Thakkar *et al.*, 2008) and with the number of tracts in the forceps minor (Thomas *et al.*, 2011). More severe social-communicative deficits have been correlated with reduced FA in fronto-striatal regions, temporal regions, the posterior part of the corpus callosum (Cheung *et al.*, 2009), left and right uncinate fasciculus, left superior

longitudinal fasciculus, left and right fornix (Poustka *et al.*, 2011), the dorsolateral prefrontal cortex (Noriuchi *et al.*, 2010), right anterior thalamic radiation and right uncinate fasciculus (Cheon *et al.*, 2011) and left cerebellar peduncle (Catani *et al.*, 2008) and with increased fibre length and density in the corpus callosum (Kumar *et al.*, 2010). Some studies however have found no relationship between ASD symptomatology and white matter measures (Alexander *et al.*, 2007a; Barnea-Goraly *et al.*, 2010; Shukla *et al.*, 2010a; Hong *et al.*, 2011; Jou *et al.*, 2011a). Reduced microstructural integrity of white matter in autism has also been correlated with lower IQ scores (Alexander *et al.*, 2007a) and with increased response times in a pictorial problem-solving task (Sahyoun *et al.*, 2010a).

A number of studies have also investigated the behavioural consequences of functional connectivity abnormalities in ASD and have reported correlations between altered functional connectivity and core symptoms of autism. Reduced fronto-posterior functional connectivity was found to correlate with increased severity of autism (as measured by total ADOS score) (Just *et al.*, 2007). Poorer social functioning in individuals with ASD has been associated with reduced functional connectivity between the superior frontal gyrus and posterior cingulate (Monk *et al.*, 2009; Weng *et al.*, 2010) and communication deficits have been associated with increased functional connectivity between regions of the default mode network during the resting state (Weng *et al.*, 2010). Increased severity of repetitive behaviours in autism has been associated with reduced functional connectivity between frontal structures and the posterior cingulate (Weng *et al.*, 2010), and also with increased functional connectivity between the posterior cingulate and parahippocampal gyrus (Monk *et al.*, 2009) and between the anterior cingulate and frontal eye fields (Agam *et al.*, 2010). In summary, results from both anatomical and functional studies suggest that disrupted neural connectivity in autism impacts negatively on core features of the condition.

1.2. Evidence for a relationship between disrupted brain white matter structure and functional connectivity abnormalities in ASD

The direct impact of white matter abnormalities on functional connectivity in ASD has been less well studied. This is surprising given the extensive literature that has documented abnormal functional connectivity in the disorder, and the lack of knowledge about the pathophysiology of this abnormal functional connectivity. A small number of studies have used a measure of the size of the corpus callosum as an index of "anatomical connectivity" and demonstrated a relationship between reduced size of the corpus callosum and reduced functional connectivity during a number of neuropsychological paradigms, including the Tower of London task (Just *et al.*, 2007), a sentence comprehension and visual imagery task (Kana *et al.*, 2006), the Embedded Figures task (Damarla *et al.*, 2010), a narrative comprehension task (Mason *et al.*, 2008) and whilst participants were at rest (Cherkassky *et al.*, 2006). No previous studies in autism have attempted to identify whether there are abnormal white matter connections that directly link brain regions showing abnormal functional connectivity, nor have any studies examined the relationship between alterations in structural connectivity and functional connectivity.

1.3. Evidence for a relationship between brain white matter structure and brain activation in neurotypical populations

In neurotypical populations, multimodal (functional MRI and diffusion MRI) imaging studies investigating the white matter correlates of brain activation and information processing have demonstrated important relationships between brain white matter structure, functional brain activation and behaviour. Typically, the methodological approach used to integrate functional brain activation data with structural diffusion imaging data involves using fMRI activation loci as seeds for diffusion tractography. This method results in isolation of white matter tracts that connect the functionally defined regions and are specifically relevant to the functional system of interest.

Structural connectivity in the visual, motor and language systems has been probed using such fMRI-guided seed regions for tractography. Structural

connections in the visual cortex were isolated using seed regions for diffusion tractography. These seed regions were generated from functional activation maps during visual stimulation and visual motion tasks (Kim and Kim, 2005; Lanyon *et al.*, 2009). More recently a similar multimodal approach was used to define the structural cortical network involved in saccadic eye movement control in humans (Anderson *et al.*, 2012). Structural connections between striatum and primary motor cortex have been extracted using functionally defined seed regions for tractography that were generated during motor tasks (Guye *et al.*, 2003; Staempfli *et al.*, 2008). Anatomical pathways linking functionally specified language areas have also been isolated using this method (Saur *et al.*, 2008).

Importantly, some studies using this fMRI-guided approach to tractography have also examined correlations between DTI, fMRI and behavioural (RT) data in order to better characterize relationships between brain structure, function and behaviour. Higher FA values of fibres in the splenium of the corpus callosum were associated with a greater BOLD response in the visual cortex following visual stimulation (Toosy *et al.*, 2004). The authors suggested that functional activity within brain regions was constrained by the structural properties of the white matter tracts connecting those regions. During an object recognition task, increased FA in the splenium of the corpus callosum was associated with faster RTs and reduced BOLD response, whereas increased FA in the genu of the corpus callosum was associated with longer RTs and increased BOLD response (Baird *et al.*, 2005). These results suggest that behavioral performance on a task is related to cortical activity in multiple regions, as well as to the integrity of the fibers connecting them (Baird *et al.*, 2005). Strong correlation between FA values in frontoparietal white matter and BOLD response in frontoparietal grey matter during a working memory task has been identified (Olesen *et al.*, 2003), and a study investigating hemispheric asymmetries in language-related pathways reported a positive correlation between increased lateralization of fMRI activation and increased mean FA in language related pathways (Powell *et al.*, 2006).

Taken together, the results of these multimodal imaging studies provide evidence for a relationship between brain white matter structure, functional brain activation and behaviour in neurotypical populations.

1.4. Evidence for a relationship between brain white matter structure and functional connectivity in neurotypical populations

The integration of functional and structural connectivity analysis in the same study population allows for the investigation of inter-participant variability in structural connectivity and provides an opportunity to relate this variability to differences in individual functional connectivity and behaviour (Hagmann *et al.*, 2008).

Studies that have adopted this approach in typically developing individuals have shown that there is evidence of substantial correspondance between structural and functional connectivity. A number of studies that have combined analyses of structural and functional connectivity during the resting state have revealed a structural basis for resting state functional connectivity. Regions of the default mode network are linked by white matter tracts (Greicius *et al.*, 2009), the level of white matter organization in the cingulum is correlated with resting state functional connectivity between midline brain regions (van den Heuvel *et al.*, 2008) and maps of resting state functional connectivity in adult macaque monkeys were shown to be markedly similar to maps of structural connectivity obtained from tracer studies (Vincent *et al.*, 2007; Margulies *et al.*, 2009). These combined structural/resting state studies have also provided evidence for a relationship (albeit complex) between anatomical and functional connectivity. Studies that have generated whole brain maps of functional and anatomical connectivity on the same cohort of participants have demonstrated that structural connection patterns and functional interactions between regions of the cortex are significantly correlated (Hagmann *et al.*, 2008; Skudlarski *et al.*, 2008; Honey *et al.*, 2009). This relationship between structural and functional connectivity is not a simple one however; strong functional connections often exist between regions with no direct anatomical connection (Honey *et al.*, 2009). Such functional connections may arise from

indirect white matter connections, or from the two regions receiving common input from a third region (Behrens and Sporns, 2012).

There is also some evidence supporting a direct relationship between anatomical and functional connectivity at the level of individual pathways. The strength of functional connectivity within the default mode network was positively correlated with the level of white matter organization in the cingulum, as estimated by FA (van den Heuvel *et al.*, 2008). Consistent with the hypothesis that functional connectivity is directly related to anatomical connectivity, a study of three patients with callosal agenesis revealed reduced interhemispheric functional connectivity in the motor and auditory cortices (Quigley *et al.*, 2003). Complete section of the corpus callosum in a young boy with intractable epilepsy resulted in a striking loss of interhemispheric resting state functional connectivity, with preservation of intrahemispheric functional connectivity (Johnston *et al.*, 2008). In patients with multiple sclerosis, disease-related reduction of functional connectivity between left and right primary sensorimotor cortices was associated with increased radial diffusivity in the white matter tracts connecting these regions, again indicating a relationship between reduced anatomical connectivity and reduced functional connectivity (Lowe *et al.*, 2008).

In summary, studies investigating a link between structural and functional connectivity in neurotypical populations have indicated that functional connectivity has a structural basis, and that there is evidence of substantial correspondence between the strength of structural and functional connectivity.

1.5. Aim and hypothesis

In ASD, there is strong evidence for disrupted functional and anatomical connectivity but no previous studies have attempted to integrate these types of connectivity analyses. As discussed above, such multimodal connectivity studies in neurotypical populations have revealed strong relationships between white matter organization, functional connectivity and behaviour. Thus the aims of this study were twofold; first to investigate the structural integrity of white matter that directly connected brain regions showing

abnormal functional connectivity in ASD, and second to investigate relationships between brain white matter structure, functional connectivity and behaviour. To do this, brain regions from the functional connectivity analysis were used as seed regions for diffusion tractography in order to isolate white matter tracts that directly linked the two regions. Microstructural organization of these white matter tracts was assessed and correlated with both functional connectivity and behavioural measures to provide a comprehensive examination of the relationships between brain structural connectivity, functional connectivity and behaviour in ASD. It was hypothesized that there would be white matter tracts linking some, but not all, pairs of brain regions that showed abnormal functional connectivity. This hypothesis was based on the knowledge that functional connectivity between regions does not always require a direct white matter connection, but can be mediated by indirect connections or input from unrelated regions (Behrens and Sporns, 2012). It was also hypothesized that white matter structure would be abnormal in tracts directly connecting the functionally defined seed regions and that there would be correlation between microstructural organization of white matter, functional connectivity and behaviour.

2. Methods

2.1. Participants and MRI datasets

As described in chapter 2, section 2, 22 individuals with ASD and 22 age- and IQ-matched controls completed a mental rotation task during functional MRI scanning. Psychophysiological interaction (PPI) functional connectivity analysis was performed on this data as described in chapter 3 section 2.1. Details of participants, the mental rotation paradigm, functional MRI image acquisition, behavioural analysis, functional MRI analysis and PPI functional connectivity (fcMRI) analysis are all outlined in chapter 2, section 2 and chapter 3 section 2.1. Whole brain High Angular Resolution Diffusion Imaging (HARDI) data were acquired for each of these participants and the data was pre-processed using the protocols outlined in chapter 4, sections 2.2 – 2.3. Demographics of participants are outlined in Table 1.

Table 1: Demographics of study participants

	Control	ASD	p-value
Number	22	22	
Gender	Male (22)	Male (22)	
Age Mean (SD, Range)	17.51 (2.76, 13.6-21.3)	17.56 (2.91, 13.0-21.8)	0.76
Full scale IQ* Mean (SD, Range)	110.50 (16.97, 84-147)	105.95 (13.46, 84-127)	0.25
Verbal IQ^ Mean (SD, Range)	117.60 (12.69, 93-134)	105.60 (18.48, 79-134)	0.11
Performance IQ- Mean (SD, Range)	120.80 (26.39, 91-155)	124.35 (17.46, 99-155)	0.73
Handedness	Right (22)	Right (22)	
Medication	None	None	
Ethnicity	Irish (22)	Irish (22)	

*Full scale IQ was estimated based on four sub-scales of the WISC/WAIS (Wechsler Intelligence Scale for Children (WISC-III or IV UK), (Wechsler, 1991, 2004) and Wechsler Adult Intelligence Scale (WAIS-III), (Wechsler, 1997).

^Verbal IQ was estimated using the Information and Vocabulary subtests of the WISC III for n=10 participants with ASD and n=10 matched controls, using Sattler's method (Sattler, 1992). It was not possible to produce a Verbal IQ for n=12 participants with ASD and n=12 controls, as the WISC IV was used to estimate full scale IQ for these participants.

~ Performance IQ was estimated using the Picture Completion and Block Design subtests of the WISC III for n=10 participants with ASD and n=10 for controls, using Sattler's method (Sattler, 1992). It was not possible to produce a Performance IQ for n=12 participants with ASD and n=12 controls, as the WISC-IV was used to estimate full scale IQ for these participants.

2.2. Selection of functionally defined seed regions for diffusion tractography

To investigate the links between brain structural connectivity and brain functional connectivity, fcMRI data and diffusion MRI data from the same participants were combined. Three questions were posed. First, were there white matter tracts that directly linked brain regions with abnormal functional connectivity in ASD? Second, if white matter tracts directly linking brain regions of abnormal functional connectivity were identified, were there structural abnormalities of this white matter? Third, was functional connectivity associated with structural integrity of white matter? Results of the functional connectivity analysis, discussed in detail in Chapter 3 (sections 3 and 4), revealed that there were significant group differences in functional connectivity between numerous brain regions. In the current study, pairs of brain regions showing abnormal functional connectivity in ASD were used as seed regions of interest (ROIs) for diffusion tractography analysis. To rationalize the number of analyses, only ipsilateral pairs of brain regions that showed abnormal functional connectivity were selected for analysis (i.e. the pair of brain regions had to be either in right or left hemisphere). The analysis was limited to ipsilateral pairs as it was thought less likely that there would be direct long-range white matter connections between left and right hemispheres. For example, unless the two regions showing abnormal connectivity both were in the region of the corpus callosum, it was unlikely that there would be one direct white matter tract linking them. In total there were sixteen ipsilateral pairs of brain regions that showed abnormal functional connectivity in ASD. These are shown shaded in Table 2, which is a modified version of Table 3, chapter 3, in which the results of between-group differences in functional connectivity are summarised.

Seed ROI			Cluster location	BA	Voxels	X	Y	Z	Effect
Right BA8	Frontal	L	Medial Frontal Gyrus	6	2024	1.6	-4.5	52.3	C - ASD +
		L	Inferior Frontal Gyrus	47	1154	22	-30.4	2.2	C - ASD +
		L	Superior Frontal Gyrus	11	253	23.6	-44	-18.2	C - > ASD -
		L	Inferior Frontal Gyrus	47	187	50.2	-25.8	-7	C + > ASD+
		R	Superior Frontal Gyrus	11	162	-27	-42.6	-16.6	C - ASD +
	Parietal	L	Precuneus	7	673	27.4	55.7	51.5	C - ASD +
	Cingulate	L	Posterior Cingulate	23	331	1.1	32	23.1	C - ASD +
	Temporal	L	Superior Temporal Gyrus	38	461	34.9	-15.9	-21.9	C - ASD +
		L	Fusiform Gyrus		277	28.9	58.1	-9.5	C - ASD +
	Occipital	L	Cuneus	19	1330	18.7	85.3	30.1	C - ASD +
			Cuneus	17	222	1.4	91.8	2.8	C - ASD +
	Cerebellum	L	Culmen		244	10.1	48.2	-0.1	C - ASD +
	Subcortical	L	Thalamus		1106	1.4	19.8	9.6	C - ASD +
Right Fusiform	Frontal	R	Precentral Gyrus	4	170	-32	21.5	60.6	C - > ASD -
	Parietal	L	Postcentral Gyrus	1	346	33.8	33.9	66.4	C - ASD +
	Temporal	L	Parahippocampal Gyrus	20	162	43.2	14.7	-15.7	C - > ASD -
	Subcortical	L	Pons		264	8.9	16.7	-35.2	C - ASD +
Right Cuneus	Parietal	L	Inferior Parietal Lobule	40	404	55.5	53.9	39	C + > ASD +
		R	Inferior Parietal Lobule	40	222	-58.1	32.2	28.2	C + ASD -
		R	Supramarginal Gyrus	39	180	-53.8	58	30.1	C + > ASD +
	Temporal	L	Superior Temporal Gyrus	39	1439	51.2	61.2	20.5	C + ASD -
		R	Middle Temporal Gyrus	22	720	-52	39.1	5.4	C + ASD -
Left BA19	Frontal	R	Middle Frontal Gyrus	6	977	-18.3	10	59.1	C - > ASD -
		R	Medial Frontal Gyrus	6	935	-1.8	-3.6	51.5	C - > ASD -
	Parietal	L	Postcentral Gyrus	3	541	46.2	15.4	52.2	C - > ASD -
	Temporal	L	Uncus	20	165	28.3	-0.1	-33.1	C - ASD +
	Occipital	L	Cuneus	18	657	19.2	84.4	26.1	C - > ASD -
		R	Cuneus	17	459	-4.9	91.5	4.7	C - ASD +
		R	Cuneus	18	177	-16.1	86.8	19.2	C - ASD +
	Subcortical	L	Caudate Head		1198	10.2	-7	4.7	C - > ASD -
		L	Thalamus		1026	4.6	23.1	8.9	C - > ASD -
		L	Caudate Body		337	24.5	18.3	28.9	C - ASD +
		R	Caudate		598	-13.4	-13.6	12.1	C - > ASD -
		R	Lentiform		233	-17.6	-4.2	-4.6	C - > ASD -
Left Inferior Semilunar Lobule	Frontal	L	Precentral Gyrus	6	347	48.4	-2.3	34.4	C - > ASD -
		R	Middle Frontal Gyrus	6	653	-27.5	7.4	48.5	C - > ASD -
	Parietal	L	Precuneus	7	255	17.2	77.1	45.7	C - > ASD -
		L	Superior Parietal Lobule	7	173	25.3	59.9	55.7	C - > ASD -
		R	Superior Parietal Lobule	7	325	-22.9	65.1	46.4	C - > ASD -
	Occipital	L	Cuneus	19	566	21.3	88.2	25.4	C - > ASD -
Right Caudate	Temporal	R	Superior Temporal Gyrus	22	317	-62.2	44.4	6.2	C + ASD -
	Cerebellar	L	Cerebellar Tonsil		380	34.9	35.6	-44.1	C + > ASD +
		L	Cerebellum (XIII)		347	41.8	50.6	-51.9	C + > ASD +
	Subcortical	L	Thalamus		218	6.1	18.7	8	C - > ASD -

Table 2: Location and size of clusters showing a significant main effect of Group in PPI analysis for each seed region of interest. Grey shading indicates ipsilateral brain region pairs that were used as seed ROIs for tractography. [Direction of between-group difference denoted with arrows (- = Negative connectivity, + = Positive connectivity), > = greater than (note that when both groups show negative connectivity the > means a larger negative value), C = Control group, ASD = Autism Spectrum Disorder group].

2.3. Preparation of functionally defined seed regions for tractography

To prepare these sixteen pairs of seed ROIs for tractography, each cluster of interest was isolated from the fcMRI analysis and was projected back from standard MNI space into the space of the original subjects' diffusion data (native space) using the FSL TBSS Deproject tool (http://www.fmrib.ox.ac.uk/fsl/tbss/index.html). Seed ROIs were required to be in native space to allow a constrained spherical deconvolution (CSD) based tractography approach (discussed in chapter 5, section 1.3). Three of these seed regions (namely, the right superior frontal gyrus, right superior temporal gyrus and left inferior semilunar lobule) failed to deproject successfully into native space. This failure to deproject occurred because the fcMRI clusters fell solely within the external extremity of the grey matter cortex and did not extend into subcortical white matter regions. These subcortical regions are used by (and required by) the TBSS analysis during construction of the FA skeleton that is needed for registration transformations between native and standard space. Therefore after deprojection, there were ten pairs of brain regions in native diffusion space that were used as seed ROIs for CSD-based tractography. To identify white matter tracts that ran directly between each pair of seed ROIs, the two regions were coded as "AND" regions (i.e. only white matter tracts that passed through ROI 1 *and* ROI 2 were isolated) by binarising the masks (see image (a) in Figures 1-5). CSD was used to extract the fibre orientation distribution (FOD) from the diffusion weighted signal in each voxel (Tournier *et al.*, 2007). The white matter tracts between each pair of seed ROIs were reconstructed based on this FOD (Jeurissen *et al.*, 2011). The fibre-tracking algorithm is based on the estimated FOD obtained from CSD (Jeurissen *et al.*, 2011) (see chapter 5 section 2.2. for details).

Preliminary tractography analysis was carried out using the exact clusters from functional connectivity analysis as outlined in Table 2, however this analysis indicated that there were no (or very very few) white matter tracts between the pairs of brain regions. One possible reason for this is that these are functionally defined ROIs, which are essentially generated by BOLD

signal fluctuation in grey matter, and therefore they may not have projected far enough into the adjacent white matter. A pragmatic approach was thus adopted in which the whole analysis was re-run using larger ROIs to increase projection into white matter. These larger ROIs were created in standard space by generating a sphere 8 x 8mm for each cluster with its centre-point the centre of mass of the original fcMRI derived cluster. This size of sphere was chosen as it corresponded with the size of the seed spheres used in the original functional connectivity analysis. These spheres were then back projected from standard MNI space to each participants' native diffusion space and used as seed ROIs for CSD based tractography as outlined above.

2.4. Dependant measures

For each tract in each participant, microstructural measures of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD), the Westin measures of linear diffusion co-efficient (CL) and planar diffusion co-efficient (CP) (Westin *et al.*, 2002) and a macrostructural measure of tract volume were computed from the tracts (Lebel *et al.*, 2008).

2.5. Statistical analysis

2.5.1. Between-group differences in white matter structure

Statistical comparisons of the data were performed using PASW (SPSS) software version 18 (SPSS Inc., Chicago, IL). For all analyses the level of statistical significance was defined as $p<0.05$ (two-tailed) and Bonferroni corrections were used throughout the analyses. To investigate whether there were between-group differences in the white matter of tracts that directly connected a pair of brain regions, univariate ANOVA with Group (ASD / Control) as the between-subjects factor was performed for the dependant measures FA, MD, RD, AD, CP, CL and tract volume in each separate set of these white matter tracts.

2.5.2. Correlation analyses

To explore how brain white matter structure, functional connectivity and behaviour are related, a number of exploratory correlation analyses (using bivariate Pearson and Spearman correlation analyses) were used to

investigate the relationships between 1. white matter structure and functional connectivity, 2. white matter structure and behaviour, 3. functional connectivity and behaviour. Given the extremely limited data, in both autism and healthy populations, on relationships between brain structural connectivity, functional connectivity and behaviour, it was felt important that all these measures were included to comprehensively explore these possible associations.

Measures of white matter structure, functional connectivity and behaviour included in the correlation analyses

For white matter structure, four measures of white matter microstructural integrity (FA, ADC, RD, AD) were included in the correlation analyses. These are the most commonly reported measures of white matter organisation in the literature.

Four measures of functional connectivity were included in the correlation analyses; negative functional connectivity on Same trials, negative functional connectivity on Mirror trials, positive functional connectivity on Same trials and positive functional connectivity on Mirror trials. In order to explain the rationale for dividing functional connectivity measures into these four groups, a brief summary of the behavioural and PPI analyses of the mental rotation data is outlined below. Behavioural analysis of the mental rotation task revealed an intriguing dissociation in mean response times between groups whereby the control group slowed significantly on the more cognitively challenging Mirror trials relative to Same trials, but the ASD group did not show this slowing and instead performed the two trial types at similar speeds. Functional connectivity analysis demonstrated that not only were there significant overall between-group differences in functional connectivity, but there was also a dissociation in patterns of connectivity, which was characterised by differing patterns of connectivity between the Groups on Same and Mirror trials. Therefore, as behavioural and functional connectivity analyses both demonstrated interesting between-group differences on Same and Mirror trials, it was felt important to include this Same/Mirror distinction in the current correlation analyses. A distinction was also made between negative and positive functional connectivity for these

correlation analyses. The PPI functional connectivity analysis revealed patterns of negative and positive functional connectivity between different pairs of brain regions. Negative connectivity indicates that the influence the task has on activity in the seed region produces a correlated opposite effect on the projection region, which is consistent with (but not proof of) one region suppressing the other. In contrast, positive connectivity between a pair of brain regions indicates that as activity in one brain region increases, there is a correlated increase in activity in the other region. Many studies of functional connectivity simply report the strength of connectivity without considering the direction of functional coupling. In terms of the functional interactions between brain regions however, there is an important difference between positive and negative functional connectivity and it was felt important to also include this negative/positive distinction in these correlation analyses. Functional connectivity values were individual z scores extracted from the PPI Main effect of Group results (see chapter 3 section 3.1). These were extracted for each participant for each pair of brain regions in which there were white matter connections.

Two measures of behaviour were used in the correlation analyses. First, two measures of autism severity were estimated using the ADOS score for communication/social interaction and the ADOS score for stereotyped behaviours/restricted interests. Second, a measure of visuospatial processing speed was calculated using mean response times during mental rotation (details of the behavioural analysis of the mental rotation task are outlined in chapter 2, section 3.1.1). As described above, there were interesting between-group differences in response times on Same and Mirror trials, thus data for correlation analyses were divided into mean response times on Same and Mirror trials.

A set of correlation analyses was performed for every set of brain regions that had direct white matter connections.

3. Results

Table 3 summarises the results of the tractography analyses between the ten pairs of seed regions that showed abnormal functional connectivity. In summary, there were white matter tracts directly connecting pairs of seed regions in five of the analyses; between left BA19 and left uncus, left BA19 and left cuneus, left BA19 and left caudate head, left BA19 and left caudate body and left BA19 and left thalamus (see Figures 1-5). There were no white matter tracts in any participants between the other five pairs of seed regions. The results section below outlines functional connectivity, white matter and correlation analysis results for each of the five pairs of seed regions between which there were white matter connections.

ROI 1	X	Y	Z	ROI 2	X	Y	Z	Functional Connectivity	Tractography result
Right Fusiform	-55	51	-19	Right Precentral Gyrus	-32	22	61	C - > ASD -	No direct tracts
Right Cuneus	-11	75	6	Right Inferior Parietal Lobule	-58	32	28	C + ASD -	No direct tracts
				Right Supramarginal Gyrus	-54	58	30	C + > ASD +	No direct tracts
				Right Middle Temporal Gyrus	-52	39	5	C + ASD -	No direct tracts
Left BA19	42	73	-2	Left Postcentral Gyrus	46	15	52	C - > ASD -	No direct tracts
				Left Uncus	28	0	-33	C - ASD +	Tracts in 5 controls, 6 ASD
				Left Cuneus	19	84	26	C - > ASD -	Tracts in 22 controls, 22 ASD
				Left Caudate head	10	-7	5	C - > ASD -	Tracts in 9 controls, 11 ASD
				Left Thalamus	5	23	9	C - > ASD -	Tracts in 12 controls, 13 ASD
				Left Caudate body	25	18	29	C - ASD +	Tracts in 23 controls, 23 ASD

Table 3: Summary of results from functional connectivity and tractography analysis. ROI_1 and ROI_2 refer to the seed regions used in tractography analysis. [Direction of between-group difference for functional connectivity is denoted with arrows (- = Negative connectivity, + = Positive connectivity), > = greater than (note that when both groups show negative connectivity the > means a larger negative value), C = Control group, ASD = Autism Spectrum Disorder group].

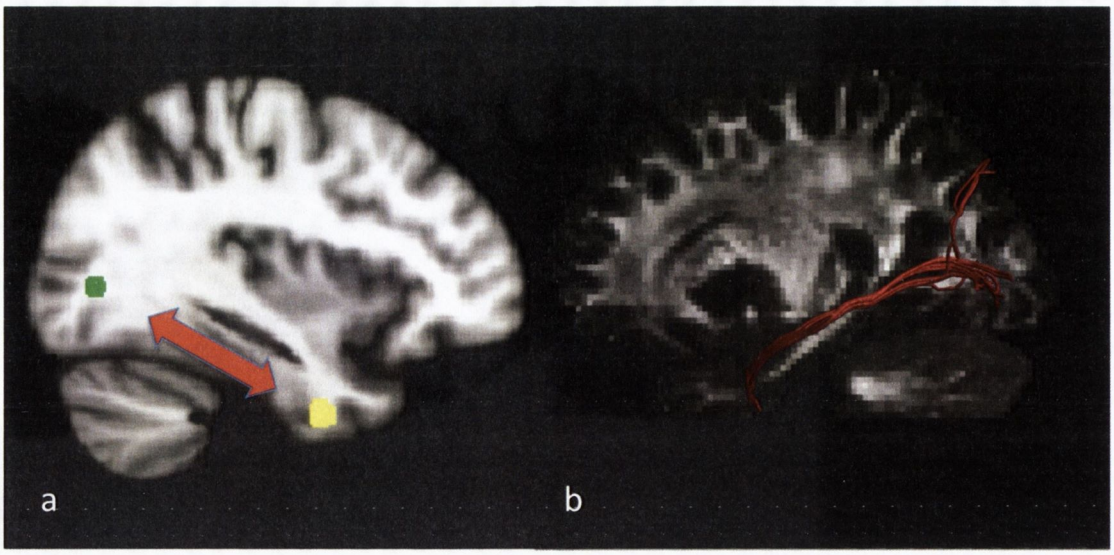


Figure 1: (a) Seed regions for tractography in left BA19 (green sphere) and left uncus (yellow sphere). The ASD group showed reduced functional connectivity relative to controls between these regions during a mental rotation task. (b) Example of white matter tracts that run through the left BA19 and left uncus seed regions in one participant.

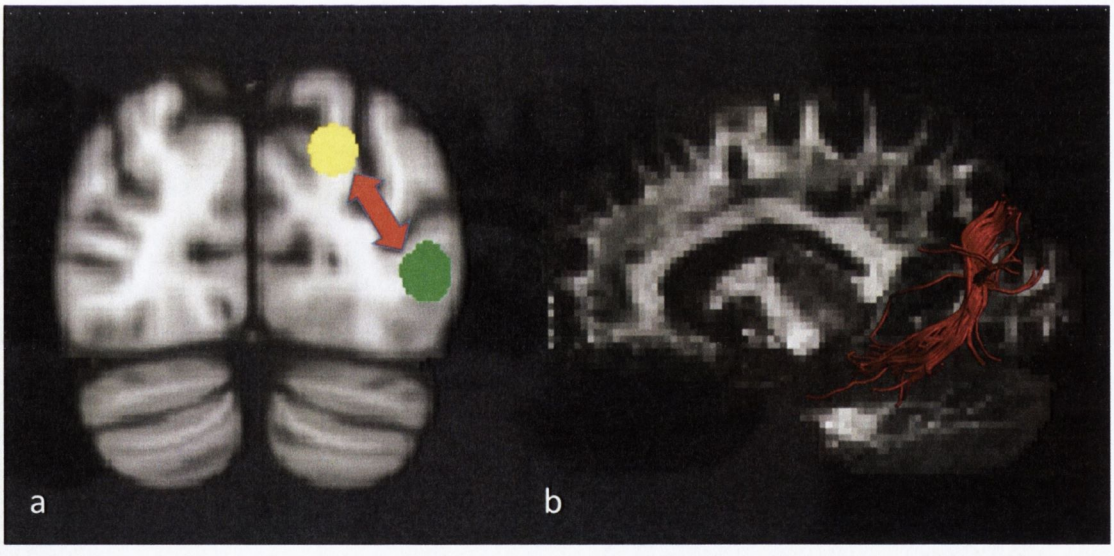


Figure 2: (a) Seed regions for tractography in left BA19 (green sphere) and left cuneus (yellow sphere). The ASD group showed reduced functional connectivity relative to controls between these regions during a mental rotation task. (b) Example of white matter tracts that run through the left BA19 and left cuneus seed regions in one participant.

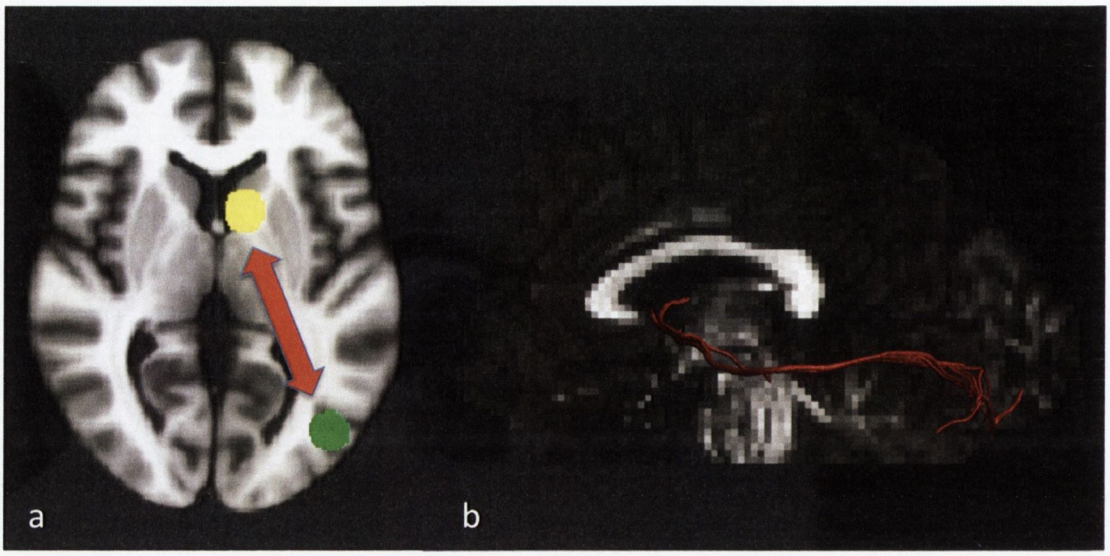


Figure 3: (a) Seed regions for tractography in left BA19 (green sphere) and left caudate head (yellow sphere). The ASD group showed reduced functional connectivity relative to controls between these regions during a mental rotation task. (b) Example of white matter tracts that run through the left BA19 and left cuneus seed regions in one participant.

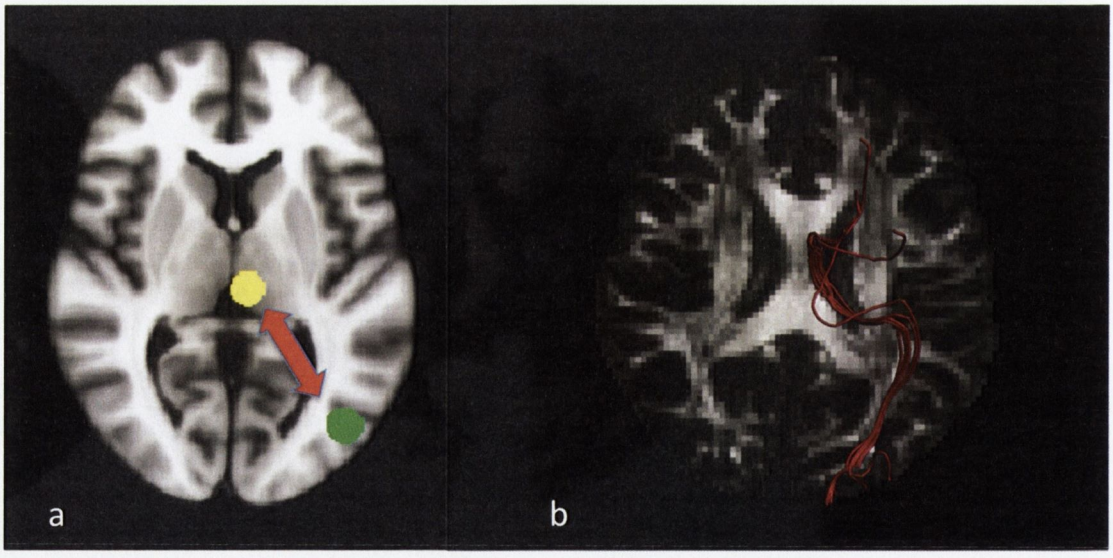


Figure 4: (a) Seed regions for tractography in left BA19 (green sphere) and left thalamus (yellow sphere). The ASD group showed much weaker functional connectivity relative to controls between these regions during a mental rotation task. (b) Example of white matter tracts that run through the left BA19 and left thalamus seed regions in one participant.

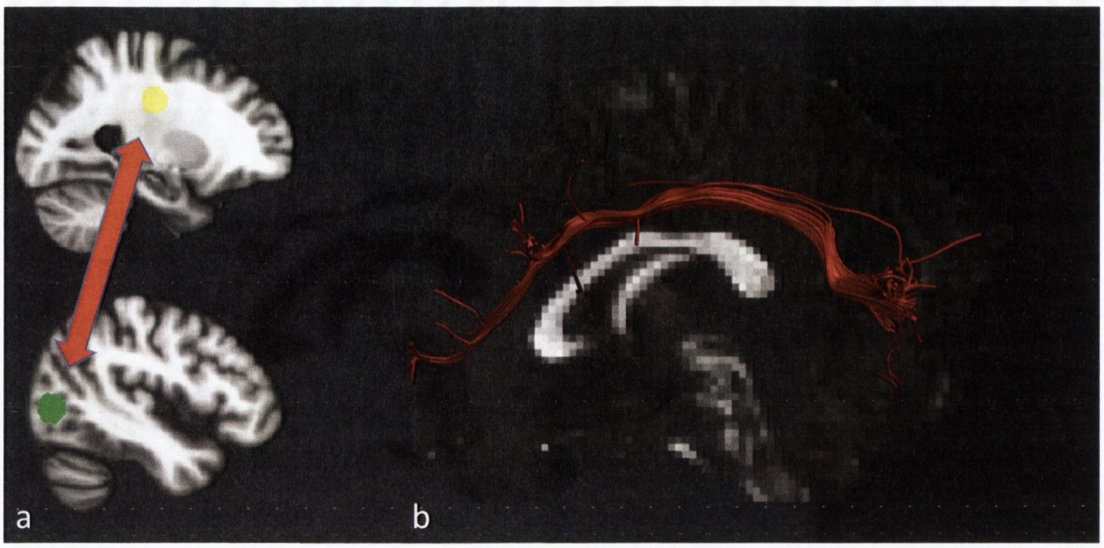


Figure 5: (a) Seed regions for tractography in left BA19 (green sphere) and left caudate body (yellow sphere). The ASD group showed increased functional connectivity relative to controls between these regions during a mental rotation task. (b) Example of white matter tracts that run through the left BA19 and left caudate body seed regions in one participant.

3.1. Connectivity between left BA 19 and left uncus

3.1.1. Functional connectivity between left BA19 and left uncus

There was a significant between-group difference in functional connectivity between left BA19 and the left uncus (see Table 2). Controls showed negative functional connectivity between these regions whereas the ASD group showed much weaker, slightly positive functional connectivity (mean ASD 0.005 , mean controls -0.020).

3.1.2. White matter tracts between left BA19 and left uncus

There were white matter tracts directly connecting the seed regions in left BA19 and the left uncus in only 5 controls and 6 participants with ASD (see Table 3). This white matter appeared to be part of the left inferior fronto-occipital fasciculus / left inferior longitudinal fasciculus (see Figure 1). There were no between-group differences in white matter micro- or macrostructure in this tract (see Table 4), and no significant correlations between diffusion measures and behaviour (see Table 6). Power was limited however, by the small sample size. There was insufficient data to perform correlation analyses of functional connectivity and diffusion measures or functional connectivity and behavioural measures.

3.2. Connectivity between left BA19 and left cuneus

3.2.1. Functional connectivity between left BA19 and left cuneus

There was a significant between-group difference in functional connectivity between left BA19 and left cuneus (see Table 2). There was strong negative connectivity in the control group whereas the ASD group showed very weak negative connectivity between these regions (mean ASD -0.010, mean controls -0.066).

3.2.2. Changes in white matter between left BA19 and left cuneus

There were white matter tracts directly connecting the seed regions in left BA19 and left cuneus in 22 controls and 22 participants with ASD (see Table 3). This white matter tract ran intra-occipitally in the left hemisphere (see Figure 2). There were no between-group differences in micro- or macrostructural integrity of this tract (see Table 4).

3.2.3. Correlation analyses of behavioural measures, diffusion measures and functional connectivity in left BA19/left cuneus region

Controls

In controls, there was a positive correlation between the behavioural measure of mean response time (MRT) during Mirror trials and the diffusion measures of ADC (r 0.452, $p<0.035$) and RD (r 0.499, $p<0.018$) (see Table 6). These correlations indicated that reduced white matter organization in this tract (as RD and ADC increase) was associated with increased MRT (i.e. slower performance of mental rotation). There were no other significant correlations between the functional connectivity and mean response time data (Table 5), or between the diffusion measures in the white matter tracts and FC during the mental rotation task (Table 7) for the control group.

ASD

For the ASD group, no significant correlations were found between the FC and mean RT measures (Table 5), the microstructural diffusion measures and the mean RTs (Table 6), or the diffusion measures in the white matter tracts and the FC (Table 7). There was a significant correlation between positive functional connectivity during Same trials and ADOS communication and social interaction scores ($p<0.001$, r_s -0.986) indicating that an increase in positive functional connectivity was associated with reduced severity of this ADOS score.

3.3. Connectivity between left BA19 and left caudate head

3.3.1. Functional connectivity between left BA19 and left caudate head

There was a significant between-group difference in functional connectivity between left BA19 and left caudate head (see Table 2), which was characterized by stronger negative connectivity in the control group compared with the ASD group (mean ASD -0.01, mean controls -0.05).

3.3.2. Changes in white matter between left BA19 and left caudate head

There were white matter tracts directly connecting the seed regions in left BA19 and left caudate head in 9 controls and 11 participants with ASD, and the white matter tracts linking these regions formed part of the left inferior fronto-occipital fasciculus (see Figure 3). Univariate ANOVA of the

dependant measures from diffusion analysis revealed that there was significantly reduced FA (mean FA ASD 0.380, controls 0.424, $F(x,x) = 5.972$, $p<0.027$, $\eta p2=0.272$) and CL (mean CL ASD 0.354, controls 0.399, $F(x,x) = 5.074$, $p<0.039$, $\eta p2=0.199$) in the ASD group relative to controls (Table 4), indicating that white matter microstructural organization was reduced in these tracts in the ASD group.

3.3.3. Correlation analyses of behavioural measures, diffusion measures and functional connectivity in left BA19/left caudate head region

Controls

In controls, there was a negative correlation between the diffusion measure of AD and the behavioural measure of MRT (r -0.694, $p<0.038$) on Mirror trials, indicating that increased AD (implying increased microstructural organization) was associated with reduced MRT (i.e. faster mental rotation performance) (see Table 5). No other significant correlations were detected.

ASD

For the ASD group, no significant correlations were found between the functional connectivity and mean RT measures (Table 5), the microstructural diffusion measures and the mean RTs (Table 6), the diffusion measures in the white matter tracts and the FC (Table 7) or ADOS scores and diffusion / functional connectivity data (Table 8).

3.4. Connectivity between left BA19 and left thalamus

3.4.1. Functional connectivity between left BA19 and left thalamus

There was a significant between-group difference in functional connectivity between left BA19 and left thalamus (see Table 2). There was strong negative connectivity in the control group whereas the ASD group showed very weak negative connectivity between these regions (mean ASD -0.003, mean controls -0.05).

3.4.2. Changes in white matter between left BA19 and left thalamus

There were white matter tracts directly connecting left BA19 and left thalamus in 12 controls and 13 participants with ASD, and this white matter appeared to comprise part of the left inferior fronto-occipital fasciculus (see

Figure 4). Univariate ANOVA revealed significantly reduced FA (mean FA ASD 0.363, controls 0.397, $F(x,x) = 4.306$, $p<0.050$, $\eta p2=0.170$), CL (mean CL ASD 0.340, controls 0.383, $F(x,x) = 8.085$, $p<0.010$, $\eta p2=0.278$) and volume (mean volume ASD 993, controls 2010, $F(x,x) = 6.255$, $p<0.021$, $\eta p2=0.230$) in the ASD group relative to controls in the white matter directly connecting left BA19 and left thalamus seed regions.

3.4.3. Correlation analyses of behavioural measures, diffusion measures and functional connectivity in left BA19/left thalamus region

Controls

In controls, there was a positive correlation between negative functional connectivity during Same trials, and mean response time during these trials (r 0.799, $p<0.003$), indicating that a reduction in MRT (i.e. faster task performance) was associated with stronger negative connectivity (see Table 5).

There were negative correlations between a number of diffusion measures and MRT; there was a negative correlation between ADC and MRT on Same (r -0.584, $p<0.046$) and Mirror (r -0.714, $p<0.009$) trials, between RD and MRT on Same (r -0.596, $p<0.041$) and Mirror (r -0.689, $p<0.013$) trials, and between AD and MRT on Mirror trials (r -0.695, $p<0.012$) (see Table 6). These results indicate that changes in microstructural organization of the white matter that connects left BA19 and left thalamus are associated with faster performance of mental rotation.

There were correlations between diffusion measures in the white matter connecting the seed regions in left BA19 and left thalamus and functional connectivity between these regions. FA and RD were correlated with negative functional connectivity on Same trials in controls. FA showed a positive correlation (r 0.685, $p<0.029$), whereas RD showed a negative correlation (r -0.687, $p<0.028$). These correlations indicate that as white matter organization increases (with increasing FA and reducing RD), there is a correlated reduction in the strength of negative functional connectivity (see Table 7).

ASD

In the ASD group, there were no correlations between mental rotation performance (MRT) and functional connectivity or white matter integrity of tracts between left BA19 and left thalamus (see Tables 5 and 6). White matter organization was correlated with functional connectivity. FA and RD were associated with positive functional connectivity in Same trials (FA r -0.915, $p<0.010$), (RD r 0.829, $p<0.041$). These results indicate that a greater level of microstructural organization in the white matter between left BA19 and left thalamus (increased FA and reduced RD) is associated with a reduction in positive functional connectivity. There was also a positive correlation between FA and the communication and social interaction score of the ADOS (r_s 0.768, $p<0.009$) (see Table 8). This result indicates that autism severity is associated with increased FA in this tract.

3.5. Connectivity between left BA19 and left caudate body

3.5.1. Functional connectivity between left BA19 and left caudate body
There was a significant between-group difference in functional connectivity between left BA19 and left caudate (see Table 2). The ASD group showed positive functional connectivity between these regions whereas controls showed much weaker, slightly negative connectivity (mean ASD 0.027, mean controls -0.004).

3.5.2. Changes in white matter between left BA19 and left caudate body
There were white matter tracts directly connecting left BA19 and left caudate body in 23 controls and 23 participants with ASD, and this white matter appeared to be part of the superior longitudinal fasciculus (see Figure 5). There were no between-group differences in micro- or macrostructural measures of the white matter linking left BA19 and left caudate body.

3.5.3. Correlation analyses of behavioural measures, diffusion measures and functional connectivity in left BA19/left caudate body region
Controls

In controls, there were no correlations between diffusion measures, functional connectivity and mean response time (MRT) during mental rotation (see Tables 5, 6, 7, 8).

ASD

In the ASD group there was a positive correlation between the behavioural measure of MRT during Mirror trials and negative functional connectivity (r 0.819, $p<0.013$) and also between MRT during Mirror trials and positive functional connectivity (r 0.525, $p< 0.017$) (see Table 5). This indicates that faster mental rotation performance on Mirror trials is associated with increased strength of negative functional connectivity and also with reduced strength of positive functional connectivity.

There were a number of correlations between diffusion measures of white matter organization and behavioural performance during mental rotation (see Table 6). There were negative correlations between FA and MRT on Same (r -0.476, $p< 0.034$) and Mirror (r -0.569, $p< 0.009$) trials and between AD and MRT on Mirror trials (r -0.461, $p< 0.041$). There were positive correlations between RD and MRT on Same (r 0.591, $p< 0.006$) and Mirror (r 0.546, $p< 0.013$) trials. These correlations indicate that as microstructural organization of the white matter linking left BA19 and left caudate body increases (characterized by an increase in FA and AD and a reduction in RD), there is an associated reduction in MRT (i.e. faster mental rotation performance) in the ASD group.

There were also correlations between diffusion measures and functional connectivity in the ASD group. FA was negatively correlated with positive functional connectivity on Mirror trials (r -0.479, $p<0.044$) and RD was negatively correlated with negative functional connectivity (r -0.878, $p<0.021$) on Same trials (see Table 7). These results indicate that increased structural organization of white matter (increased FA and reduced RD) is associated with increased strength of negative functional connectivity and also with reduced strength of positive functional connectivity.

Tract		Left BA19 – Left Uncus		Left BA19 – Left Cuneus		Left BA19 – Left Caudate head		Left BA19 – Left Thalamus		Left BA19 – Left Caudate body	
Measure	**Group**	**Mean (SD)**	**p**	**Mean (SD)**	**p**	**Mean (SD)**	**p**	**Mean (SD)**	**p**	**Mean (SD)**	**p**
FA	**ASD**	0.370 (0.057)	0.685	0.350 (0.044)	0.957	0.380* (0.039)	0.027*	0.363* (0.037)	0.050*	0.368 (0.033)	0.232
	Con	0.372 (0.070)		0.352 (0.032)		0.424* (0.051)		0.397* (0.045)		0.379 (0.035)	
ADC	**ASD**	0.00077 (0.00003)	0.865	0.00075 (0.00002)	0.494	0.00079 (0.00007)	0.647	0.00087 (0.00011)	0.774	0.00071 (0.00002)	0.802
	Con	0.00077 (0.00001)		0.00074 (0.00003)		0.00077 (0.00003)		0.00085 (0.00013)		0.00071 (0.00004)	
AD	**ASD**	0.00110 (0.00008)	0.693	0.00103 (0.00004)	0.671	0.00114 (0.00006)	0.247	0.00123 (0.00017)	0.707	0.00100 (0.00004)	0.294
	Con	0.00111 (0.00007)		0.00104 (0.00004)		0.00116 (0.00010)		0.00125 (0.00015)		0.00102 (0.00006)	
RD	**ASD**	0.00061 (0.00006)	0.926	0.00060 (0.00004)	0.619	0.00062 (0.00004)	0.187	0.00069 (0.00010)	0.454	0.00056 (0.00002)	0.691
	Con	0.00061 (0.00003)		0.00059 (0.00003)		0.00057 (0.00007)		0.00066 (0.00011)		0.00056 (0.00004)	
CP	**ASD**	0.163 (0.031)	0.646	0.169 (0.031)	0.808	0.166 (0.025)	0.929	0.162 (0.039)	0.481	0.187 (0.027)	0.851
	Con	0.173 (0.042)		0.173 (0.021)		0.164 (0.014)		0.149 (0.023)		0.187 (0.018)	
CL	**ASD**	0.345 (0.049)	0.774	0.322 (0.046)	0.962	0.354* (0.036)	0.039*	0.340** (0.042)	0.010**	0.330 (0.035)	0.262
	Con	0.344 (0.076)		0.322 (0.039)		0.399* (0.059)		0.383** (0.032)		0.341 (0.033)	
Volume	**ASD**	2037 (2666)	0.873	2955 (3589)	0.970	1494 (1025)	0.064	993* (541)	0.021*	5422 (4242)	0.542
	Con	1841 (1253)		3052 (1914)		3141 (3870)		2010* (1271)		6134 (5013)	

Table 4: The mean, standard deviation and p values for the six micro-structural dependant measures (FA, AD, RD, ADC, CP, CL) and the macro-structural measure (tract volume) for white matter tracts connecting functionally defined seed regions in the ASD and control groups. * indicates significant between-group differences with $p<0.05$ and ** $p<0.01$.

			MRT_S p (r)			MRT_M p (r)
Left BA19 – Left cuneus	FC_S -	ASD	0.533 (0.182)	FC_M -	ASD	0.553 (0.155)
		Control	0.511 (-0.177)		Control	0.933 (0.020)
	FC_S +	ASD	0.294 (-0.516)	FC_M +	ASD	0.967 (0.033)
		Control	0.916 (-0.084)		Control	^1
Left BA19 – Left caudate head	FC_S -	ASD	0.576 (-0.216)	FC_M -	ASD	0.949 (0.025)
		Control	0.071 (0.627)		Control	0.693 (0.154)
	FC_S +	ASD	^1	FC_M +	ASD	^1
		Control	^1		Control	^1
Left BA19 – Left thalamus	FC_S -	ASD	0.374 (-0.626)	FC_M -	ASD	0.830 (-0.170)
		Control	0.006 (0.797)**		Control	0.884 (0.053)
	FC_S +	ASD	0.935 (0.043)	FC_M +	ASD	0.766 (-0.157)
		Control	^1		Control	^1
Left BA19 – Left caudate body	FC_S -	ASD	0.309 (-0.503)	FC_M -	ASD	0.013 (0.819)*
		Control	0.930 (-0.027)		Control	0.534 (-0.182)
	FC_S +	ASD	0.360 (0.229)	FC_M +	ASD	0.017 (0.525)*
		Control	0.142 (0.530)		Control	0.413 (0.338)

Table 5: Results of correlation analysis between functional connectivity and mean response times during Same and Mirror trials of a mental rotation task (p: p value, r: Pearson correlation co-efficient, FC_S - Negative functional connectivity on Same trials, FC_S + Positive functional connectivity on Same trials, FC_M – Negative functional connectivity on Mirror trials, FC_M + Positive functional connectivity on Mirror trials, MRT: mean response time, ^1 Insufficient data for correlation as all functional connectivity values were negative between seed regions in left BA19 and left caudate head).

		Left BA19 – Left cuneus		Left BA19 – Left caudate head		Left BA19 – Left thalamus		Left BA19 – Left caudate body	
		MRT_S p (r)	MRT_M p (r)	MRT_S p (r)	MRT_M p (r)	MRT_S p (r)	MRT_M p (r)	MRT_S p (r)	MRT_M p (r)
FA	ASD	0.855 (-0.041)	0.439 (0.174)	0.391 (-0.305)	0.800 (-0.092)	0.979 (-0.010)	0.888 (-0.051)	0.034* (-0.476)	0.009** (-0.569)
	Con	0.534 (-0.140)	0.133 (-0.330)	0.766 (0.116)	0.394 (-0.324)	0.428 (0.253)	0.785 (0.088)	0.979 (-0.006)	0.940 (0.018)
ADC	ASD	0.734 (0.077)	0.962 (-0.011)	0.855 (0.066)	0.568 (-0.206)	0.490 (-0.248)	0.811 (-0.087)	0.091 (0.388)	0.161 (0.498)
	Con	0.102 (0.358)	0.035* (0.452)	0.065 (-0.637)	0.131 (-0.543)	0.046* (-0.584)	0.009** (-0.714)	0.352 (-0.220)	0.888 (-0.034)
AD	ASD	0.963 (0.011)	0.474 (0.161)	0.873 (-0.058)	0.514 (-0.234)	0.464 (-0.262)	0.925 (-0.034)	0.381 (-0.207)	0.041* (-0.461)
	Con	0.545 (0.136)	0.972 (0.008)	0.340 (-0.361)	0.038* (-0.694)	0.080 (-0.525)	0.012* (-0.695)	0.416 (-0.193)	0.896 (0.031)
RD	ASD	0.725 (0.080)	0.681 (-0.093)	0.702 (0.139)	0.645 (-0.167)	0.520 (-0.232)	0.750 (-.0116)	0.006** (0.591)	0.013* (0.546)
	Con	0.188 (0.291)	0.018* (0.499)	0.283 (-0.402)	0.875 (-0.061)	0.041* (-0.596)	0.013* (-0.689)	0.499 (-0.161)	0.797 (-0.062)

Table 6: Results of correlation analysis between mean response times during a mental rotation task and micro-structural diffusion measures (FA, ADC, AD RD) extracted from the white matter tracts linking functionally defined seed regions. (p: p value, r: Pearson correlation co-efficient , MRT_S; mean response time on Same trials, MRT_M; mean response time on Mirror trials)

Left BA19 – Left cuneus					
		FC_S – p (r)	FC_S + p (r)	FC_M – p (r)	FC_M + p (r)
FA	ASD	0.751 (0.093)	0.808 (-0.129)	0.703 (-0.100)	0.993 (0.007)
	Con	0.056 (-0.486)	0.107 (0.893)	0.964 (-0.011)	^1
ADC	ASD	0.318 (-0.288)	0.639 (0.246)	0.631 (-0.125)	0.527 (0.473)
	Con	0.472 (0.194)	0.867 (0.133)	0.952 (-0.014)	^1
AD	ASD	0.456 (-0.217)	0.758 (0.163)	0.281 (-0.278)	0.462 (0.538)
	Con	0.240 (-0.312)	0.412 (0.588)	0.958 (-0.012)	^1
RD	ASD	0.463 (-0.214)	0.714 (0.193)	0.981 (0.006)	0.744 (0.256)
	Con	0.098 (0.428)	0.321 (-0.679)	0.982 (-0.006)	^1
Left BA19 – Left caudate head					
		FC_S –	FC_S +	FC_M –	FC_M +
FA	ASD	0.818 (0.090)	^1	0.838 (-0.080)	^1
	Con	0.422 (0.307)	^1	0.419 (0.309)	^1
ADC	ASD	0.595 (-0.206)	^1	0.427 (-0.304)	^1
	Con	0.463 (-0.282)	^1	0.461 (-0.283)	^1
AD	ASD	0.602 (-0.202)	^1	0.368 (-0.342)	^1
	Con	0.858 (0.070)	^1	0.810 (0.094)	^1
RD	ASD	0.609 (-0.198)	^1	0.459 (-0.262)	^1
	Con	0.362 (-0.346)	^1	0.335 (-0.369)	^1
Left BA19 – Left thalamus					
		FC_S –	FC_S +	FC_M –	FC_M +
FA	ASD	0.721 (0.279)	0.010 (-0.915)**	0.601 (0.399)	0.278 (0.532)
	Con	0.029 (0.685)*	^1	0.553 (0.214)	^1
ADC	ASD	0.376 (-0.624)	0.078 (0.762)	0.907 (-0.093)	0.401 (-0.425)
	Con	0.056 (-0.619)	^1	0.638 (-0.170)	^1
AD	ASD	0.405 (-0.595)	0.195 (0.614)	0.943 (0.057)	0.465 (-0.374)
	Con	0.161 (-0.480)	^1	0.755 (-0.113)	^1
RD	ASD	0.379 (-0.621)	0.041 (0.829)*	0.846 (-0.154)	0.381 (-0.442)
	Con	0.028 (-0.695)*	^1	0.574 (-0.203)	^1
Left BA19 – Left caudate body					
		FC_S –	FC_S +	FC_M –	FC_M +
FA	ASD	0.568 (0.296)	0.564 (0.156)	0.840 (0.095)	0.044 (-0.479)*
	Con	0.280 (0.358)	0.544 (-0.234)	0.328 (0.309)	0.182 (-0.525)
ADC	ASD	0.116 (-0.707)	0.686 (0.110)	0.219 (-0.443)	0.892 (-0.032)
	Con	0.956 (0.019)	0.463 (0.281)	0.311 (-0.320)	0.975 (0.013)
AD	ASD	0.917 (-0.056)	0.569 (0.154)	0.488 (-0.317)	0.062 (-0.449)
	Con	0.476 (0.241)	0.887 (-0.056)	0.754 (0.101)	0.369 (-0.368)
RD	ASD	0.021 (-0.878)*	0.979 (-0.007)	0.650 (-0.211)	0.158 (0.347)
	Con	0.660 (-0.150)	0.485 (0.268)	0.273 (-0.344)	0.295 (0.479)

Table 7: Caption on following page

Table 7: Results of correlation analysis between diffusion measures in white matter tracts and functional connectivity during Same and Mirror trials of a mental rotation task (p: p value, r: Pearson correlation co-efficient, FC_S - Negative functional connectivity on Same trials, FC_S + Positive functional connectivity on Same trials, FC_M – Negative functional connectivity on Mirror trials, FC_M + Positive functional connectivity on Mirror trials, Con: Control, ^1 Insufficient data for correlation as all functional connectivity values were negative between seed regions in left BA19 and left caudate head).

Tract	Left BA19 – Left cuneus		Left BA19 – Left caudate head		Left BA19 – Left caudate body		Left BA19 – Left thalamus	
	ADOS C/SI r_s (p)	ADOS RB r_s (p)	ADOS C/SI r_s (p)	ADOS RB r_s (p)	ADOS C/SI r_s (p)	ADOS RB r_s (p)	ADOS C/SI r_s (p)	ADOS RB r_s (p)
FA	0.141 (0.533)	-0.130 (0.565)	0.208 (0.565)	-0.079 (0.828)	0.187 (0.429)	0.366 (0.133)	0.768 (0.009)**	0.027 (0.941)
ADC	-0.135 (0.548)	0.130 (0.563)	0.359 (0.305)	-0.184 (0.610)	0.071 (0.767)	0.145 (0.542)	-0.252 (0.483)	0.302 (0.397)
AD	-0.056 (0.804)	-0.101 (0.653)	0.302 (0.396)	0.329 (0.353)	0.358 (0.121)	0.329 (0.156)	-0.264 (0.460)	0.302 (0.397)
RD	-0.268 (0.228)	0.169 (0.452)	-0.006 (0.986)	0.158 (0.663)	-0.196 (0.407)	-0.156 (0.510)	-0.290 (0.417)	0.241 (0.502)
FC_S –	0.279 (0.333)	-0.425 (0.129)	0.068 (0.861)	-0.291 (0.448)	-0.385 (0.141)	0.369 (0.159)	^1	^1
FC_S +	-0.986 (0.0001)**	0.432 (0.392)	^2	^2	0.116 (0.827)	-0.621 (0.188)	-0.441 (0.381)	0.494 (0.320)
FC_M -	0.128 (0.625)	0.095 (0.716)	-0.037 (0.924)	0.037 (0.924)	0.783 (0.066)	0.338 (0.512)	0.775 (0.225)	-0.258 (0.742)
FC_M +	-0.632 (0.368)	0.949 (0.051)	^2	^2	-0.243 (0.549)	0.246 (0.595)	0.783 (0.066)	0.338 (0.512)

Table 8: Results of correlation analysis between ADOS scores and diffusion measures and functional connectivity measures for the ASD group. ($\mathbf{r_s}$: Spearman's correlation co-efficient, p: p-value, FC_S - Negative functional connectivity on Same trials, FC_S + Positive functional connectivity on Same trials, FC_M – Negative functional connectivity on Mirror trials, FC_M + Positive functional connectivity on Mirror trials, ADOS C/SI: ADOS communication and social interaction score, ADOS RB: ADOS repetitive behaviours score, ^1: All values identical for ADOS score, ^2 Insufficient data for correlation analysis as all functional connectivity values were negative).

4. Discussion

The main finding of this study is that there are microstructural abnormalities in white matter tracts that directly connect brain regions showing abnormal functional connectivity. In addition, there are strong correlations between measures of white matter microstructure, functional connectivity and behaviour, which provide a fascinating insight into the relationships between brain structure, brain function and information processing in both neurotypical controls and individuals with ASD.

This discussion focuses on the implications these results have for the original hypotheses of this study, which predicted firstly that there would be white matter tracts linking some, but not all pairs of brain regions showing abnormal functional connectivity, secondly that white matter structure would be abnormal in tracts directly connecting the functionally defined seed regions and finally that there would be relationships between microstructural organization of white matter, functional connectivity and behaviour.

4.1. Functional connectivity is not always associated with direct white matter connections

In this study, ten pairs of brain regions were used as seed regions for CSD based tractography. These seed regions were generated from functional connectivity maps during a mental rotation task, and indicated brain regions between which there was abnormal functional connectivity in ASD. Tractography analysis revealed that there were white matter tracts directly connecting five of these ten pairs of seed regions in most participants. For the other five seed region pairs there were no direct structural connections in any participants. This finding supports Hypothesis One.

This finding of a direct structural connection between only half of the regions showing functional connectivity is consistent with results of imaging studies that have used a similar multimodal approach of integration of functional connectivity MRI and diffusion MRI. One of the first studies to use this method reported that high functional and low structural connectivity do occur together, but that low functional connectivity rarely occurs between regions where there is high structural connectivity (Koch *et al.*, 2002). In keeping with this finding, a more recent study investigating the links between resting

state functional connectivity and structural connectivity revealed that functional connectivity between regions is not indicative of a direct structural connection between those regions (Honey *et al.*, 2009). This is likely to be because functional connectivity can be mediated by indirect connections or by input from a third region into the two regions, which modulates connectivity in the two primary regions (Koch *et al.*, 2002; Honey *et al.*, 2009; Behrens and Sporns, 2012).

4.2. Abnormal functional connectivity is associated with abnormal structural connectivity

Consistent with the prediction that abnormal functional connectivity would be associated with abnormal structural connectivity, there was reduced microstructural organization of white matter in two of the five tracts linking regions of abnormal functional connectivity. In the ASD group, there was a significant reduction in the strength of functional connectivity between an occipital seed region (left BA19) and the left caudate head and also between this occipital seed region and the left thalamus. Analysis of the diffusion measures in the white matter directly linking these occipito-striatal and occipito-thalamic regions revealed significant microstructural abnormalities in the ASD group, which were characterized by reduced FA and CL, two measures that provide an indication of the level of organization of white matter fibres. This finding of reduced structural connectivity between brain regions that also show reduced functional connectivity is particularly interesting as it provides novel evidence to suggest that structural brain pathology may contribute to the abnormal functional connectivity that has been widely reported in the autism literature. In addition, there were significant correlations between structural and functional connectivity in the occipito-thalamic region, a finding that lends strength to the hypothesis that structural and functional connectivity are interrelated, and that abnormal white matter microstructure may play an important aetiological role in aberrant functional connectivity in autism.

It is also noteworthy that the white matter in both these tracts formed part of the left inferior fronto-occipital fasciculus (IFOF), a major white matter association tract in the human brain. Interestingly, this study revealed

structural abnormalities in this sub-region of the left IFOF, whereas a previous analysis of the whole left IFOF using the same data from the same study population found no abnormalities of white matter (see chapter 5). This is of relevance as it highlights a concern that current whole brain or even tract-specific analyses of white matter may lack sensitivity in detecting white matter abnormalities, and may not be specific enough about the exact locations of pathology in cases where abnormalities are reported.

The relationship between structural and functional connectivity is however far from simple. For three of the five white matter tracts directly connecting seed regions showing abnormal functional connectivity, there was no evidence of disrupted organization of white matter. This finding is consistent with the theory that functional connectivity can be modulated by factors other than the direct structural white matter connection between regions, and it does not negate the hypothesis that structural connectivity abnormalities impact negatively on functional connectivity. In fact, further evidence for a direct relationship between structural and functional connectivity comes from the analysis of the occipito-striatal tract between left BA19 and left caudate body. Although there were no between-group differences in structural integrity of this tract, there were significant correlations between structural and functional connectivity in the ASD group, which are discussed in more detail below.

4.3. Correlations between microstructural organization of white matter, functional connectivity and behaviour

Correlation analysis revealed intriguing links between white matter microstructure, functional connectivity and behaviour in two of the five datasets in which there were direct white matter tract connections; between seed regions in left BA19 and left thalamus, and between left BA19 and left caudate.

In controls, the structure of white matter between left BA19 and left thalamus was associated with behaviour (reduced microstructural organization was associated with faster visuospatial processing), behaviour was associated with function (faster visuospatial processing was associated with stronger negative functional connectivity) and interestingly, function

was associated with structure (stronger negative functional connectivity was associated with reduced microstructural organization). Taken together, these correlation analyses suggest that during visuospatial processing, neurotypical controls benefit from increased functional inhibition between left BA19 and left thalamus, which is associated with reduced organization in white matter between these regions. In the ASD group, structural and functional connectivity in the same regions were correlated but there were no significant correlations between visuospatial processing speed and structural or functional connectivity. Therefore the effect of altered connectivity in this tract on mental rotation performance is not known. It is noteworthy however that the severity of autism was correlated with structural connectivity between left BA19 and left thalamus; increased communication and social interaction deficits in the ASD group was associated with increased microstructural organization (FA).

Functional connectivity between left BA 19 and left caudate body was significantly increased in the ASD group relative to controls, but white matter organization in the tracts directly connecting these regions was normal. The correlation analyses however implied that there were significant between-group differences in the functional use of this tract during visuospatial processing. Controls showed no association between structural, functional and behavioural measures, whereas structural connectivity, functional connectivity and visuospatial processing speed appeared to be strongly related in the ASD group. Three conclusions are drawn. Firstly, greater organization of white matter (higher FA and reduced RD) was associated with reduced strength of positive functional connectivity and with increased strength of negative functional connectivity between left BA19 and left caudate. Secondly, reduced strength of positive functional connectivity and increased strength of negative functional connectivity between these regions was associated with faster mental rotation performance. Finally, faster mental rotation performance was correlated with greater microstructural organization of the white matter between left BA19 and left caudate body. When considered together, these correlations suggest that a higher level of structural organisation of this tract confers a benefit to visuospatial

processing speed in ASD that may be mediated by increased functional suppression between left BA19 and left caudate. The lack of correlations in controls is in sharp contrast to the strong relationships found in the ASD group and might indicate that the ASD group relies on connectivity between these regions during visuospatial processing, whereas the controls do not.

Correlation analyses of connectivity and behaviour between left BA19 and left caudate head and between left BA19 and left cuneus regions did not yield such strong evidence for inter-relationship of structure, function and behaviour. Increased microstructural organization of white matter in both these tracts was associated with improved mental rotation performance in controls, possibly suggesting that these tracts may be important in visuospatial processing in a neurotypical population. There were no relationships between brain structure and functional connectivity or behaviour and functional connectivity. It is difficult to speculate on the reasons for this relative lack of structure/function/behaviour correlations in these datasets because there is very little literature documenting relationships between brain white matter structure and functional connectivity.

4.4. Direct implications of this study

Together, these findings offer a fascinating insight into the relationships between brain structure, brain function and information processing in both neurotypical controls and individuals with ASD. This multimodal imaging study has used a novel approach to integrate functional and structural neuroimaging data. It has demonstrated, for the first time, direct associations between abnormal functional connectivity and disrupted white matter structure in ASD. It also reveals that in some brain regions, individual differences in white matter organisation are related to the level of functional connectivity during a visuospatial processing task, and further, that this relationship has consequences on behaviour.

There are many studies investigating functional or structural connectivity in ASD; however none have previously attempted to relate the two types of connectivity, an approach that is vital to increase understanding of the

underlying neurobiology. The approach that is described in this study is rational and clinically feasible. It is hoped that future neuroimaging research in ASD will follow this type of methodology to integrate investigation of functional and structural connectivity. It will be interesting to see the impact of abnormal structural connectivity on functional connectivity during other neuropsychological paradigms and at rest.

4.5. Potential implications of findings from current study on therapeutic interventions for autism

A greater understanding of the specific deficits in functional and anatomical connectivity in autism is particularly salient as there is some evidence to suggest that connectivity abnormalities are amenable to training interventions. Neuroplasticity in humans is well documented (Doidge, 2007) and two fascinating studies have demonstrated training-related changes in brain white matter structure (Keller and Just, 2009; Scholz *et al.*, 2009). One study demonstrated that healthy adults who were trained on a complex visuo-spatial skill (juggling) developed an increase in FA in white matter underlying the intraparietal sulcus (Scholz *et al.*, 2009), while the other reported that after 100 hours of intensive remedial instruction, children with impaired reading ability showed an increase in FA in a brain region that, prior to instruction, had showed significantly lower FA relative to good readers (Keller and Just, 2009).

That white matter structure can be influenced by experience is highly relevant for autism research. White matter integrity is abnormal in numerous regions in autism; but it may be possible to introduce therapeutic training to stimulate improvement in white matter organization. Future treatments for autism could conceivably focus on interventions that improve white matter organization and inter-regional brain connectivity, which may lead to improvements in the behaviours that are often impaired in this condition.

4.6. Limitations

There were a number of limitations to this study. As mentioned in previous chapters, study participants are not representative for all individuals on the spectrum. In addition, it was not possible to back-project all functionally

generated seed regions into native diffusion space, and it is likely that valuable information about frontal and cerebellar white matter abnormalities was not analysed in this study as a result. For CSD-based tractography, data must be in native diffusion space. It would be possible to analyse these data using a diffusion tensor tractography approach in standard MNI space, but given the significant limitations associated with tensor based tractography that have been outlined in chapter 5, section 1.2, it was felt that this approach would not yield reliable results. In addition, we did not analyse any pairs of interhemispheric brain regions.

In an attempt to increase understanding of the relationships between brain structural connectivity, functional connectivity and behaviour, a large number of correlation analyses were carried out. Given the extremely limited data in both autism and healthy populations on the relationships between white matter structure, functional connectivity and behaviour, it was felt that it was reasonable to perform this number of exploratory correlation analyses, however it is important to point out that correction for multiple comparisons was not carried out, thus some of the significant correlations reported may have been due to chance. Nonetheless it was very interesting to note the significant inter-relatedness of structure, function and visuospatial processing speed in two of the white matter tracts investigated (between the left occipital lobe and left caudate, and left occipital lobe and left thalamus). The correlations between these three measures lend strength to the hypothesis that there are indeed relationships between structure, function and behaviour.

As mentioned earlier in the discussion, there were strong correlations between structure, function and behaviour in two datasets but a relative lack of correlation in two other datasets. A comprehensive interpretation of these findings requires more knowledge of the normal relationships between brain structure, function and behaviour. While it is unfortunate that such research has been limited, improved analytical methodologies are providing opportunities for these types of investigations to be undertaken with greater frequency in the future.

5. Conclusion

This novel multimodal imaging study reports, for the first time, direct associations between abnormal functional connectivity and disrupted white matter structure in ASD. It reveals that there are significant correlations between the microstructural integrity of white matter, functional connectivity, visuospatial processing speed and autism severity. Overall, this study adds considerably to the knowledge of how brain structure, function and behaviour are related in ASD. It is critical to understand the complex neural pathophysiology of autism in order to develop rational, targeted therapeutic interventions to improve white matter organization and increase inter-regional connectivity.

7. General Discussion

1. Introduction

Autism spectrum disorders are devastating neurodevelopmental disorders of childhood with deficits in social interaction and communication and characteristic restricted, repetitive patterns of behaviours, interest and activities. Despite decades of research, the cause of ASD remains elusive. It has a strong genetic basis with heritability estimates of greater than 90% (Bailey *et al.*, 1995; Bailey *et al.*, 1996; Hyman, 2008), however the genetics of ASD are complex and numerous susceptibility genes have been implicated in the aetiology of the disorder. A large number of these genes are known to play an important role in synaptogenesis and neural connectivity [see (Bourgeron, 2009; Gilman *et al.*, 2011)], and it has been suggested that disrupted synaptogenesis may be a core pathogenic mechanism in ASDs (Gilman *et al.*, 2011). This is particularly interesting as it supports theories of abnormal neuronal connectivity that have emerged from a decade of structural and functional neuroimaging research (Just *et al.*, 2004; Kana *et al.*, 2011). The theory of abnormal neural connectivity in ASD holds that core social and cognitive features of autism are underpinned by abnormal interregional brain connectivity and there is extensive evidence from functional MRI, structural MRI and diffusion tensor imaging studies suggesting that neural connectivity is abnormal in ASD. There is however a striking lack of research attempting to investigate the links between functional connectivity, structural connectivity and behaviour, an approach that may provide novel insights into the underlying pathophysiology of ASD.

2. Overview of thesis aims and results

The central aim of this thesis was to investigate neural connectivity in ASD from a functional and a structural perspective in order to investigate the relationships between brain structural connectivity, functional connectivity and behaviour. To achieve this aim, a multimodal (functional MRI and DTI) neuroimaging study was performed to investigate both functional connectivity and structural connectivity in individuals with ASD and neurotypical controls.

2.1. Functional MRI and functional connectivity

In the first part of this study, functional brain activity and functional connectivity were measured during a visuospatial processing paradigm of mental rotation. On a variety of behavioural tests of visuospatial cognitive function, people with ASD have been reported to demonstrate superiority over typically developing controls [e.g. (Shah and Frith, 1983; Jolliffe and Baron-Cohen, 1997; Plaisted *et al.*, 1998a; O'Riordan, 2004; Joseph *et al.*, 2009; Soulieres *et al.*, 2011)]. A number of more recent neuroimaging studies, however, have reported intact visuospatial processing from a behavioural perspective but have also shown differential brain activity and connectivity between ASD and control groups [e.g. (Ring *et al.*, 1999; Silk *et al.*, 2006; Damarla *et al.*, 2010)]. These findings suggest that visuospatial processing in autism may be accomplished in an atypical way. Mental rotation has been underinvestigated in ASD. Investigation of this cognitive function therefore provided a relatively novel approach to examining the atypicalities of visuospatial processing in the disorder. The first aim of this study was to (a) characterise the neuropsychological function of mental rotation in a well-defined sample of individuals with ASD and (b) to investigate brain activity during mental rotation in order to increase understanding of the pathophysiology of atypical visuospatial processing in ASD.

Behavioural results from this study revealed a previously unreported dissociation between ASD and control groups in response times during mental rotation. The Control group slowed significantly on the more cognitively demanding Mirror trials relative to Same trials whereas the ASD group showed consistent response times on the two trial types. This may reflect a relative superiority of processing of Mirror trials in the ASD group, a finding in keeping with the Enhanced Perceptual Functioning (EPF) theory of autism (Mottron *et al.*, 2006). Functional MRI results indicated that in the ASD group, brain activation during mental rotation was significantly reduced in frontal, parietal, occipital, temporal and cerebellar regions. Therefore individuals with ASD successfully performed the mental rotation task but with significantly reduced cortical involvement, a finding in line with previous

neuroimaging research using visuospatial paradigms in ASD. The differences in behavioural results and functional anatomy suggest that the cognitive strategies employed by the two groups during mental rotation may be different.

In order to investigate further the neural correlates of atypical visuospatial processing, a functional connectivity analysis was performed on the data. In typically developing populations, it is known that successful mental rotation relies on the integration of information within an occipital/parietal/frontal neural network (Zacks, 2007). The mental rotation paradigm selected for this study therefore provided an ideal neuroimaging opportunity to investigate functional connectivity in a spatially distributed neural network in individuals with ASD. The second question this thesis addressed was whether there was abnormal functional connectivity in the neural network required for mental rotation in ASD. Given the interesting behavioural finding of a dissociation in response times between Same and Mirror trials in ASD, this study also specifically investigated between-group differences in functional connectivity during the Same and Mirror trials of the mental rotation paradigm.

Results from the functional connectivity analyses provided a novel insight into the neural underpinnings of the behavioural results, and, on a wider scale, into the neural mechanisms of atypical visuospatial processing in ASD. There was reduced functional connectivity between multiple brain regions during mental rotation in ASD, a finding in keeping with a large body of literature. Some connections appeared to be conserved, and were recruited in a qualitatively different way by the two groups during mental rotation in this study. Atypical visuospatial processing in ASD appears to be associated with both quantitative and qualitative differences in functional connectivity, which may result in a combination of enhanced low-level perceptual processing and a reduction of higher-level cortical control.

2.2. Diffusion tensor imaging and structural connectivity

To date, the majority of functional neuroimaging studies in ASD have focused solely on functional data and have not attempted to investigate the

anatomical correlates of aberrant functional connectivity. An understanding of how brain structure impacts on functional connectivity is vital in order to further our understanding of the pathophysiology of ASD. In the next section of this thesis, DTI data from the same study population was analysed using advanced neuroimaging techniques in order to investigate whether structural connectivity was impaired in ASD. Two methodological approaches were used to examine structural connectivity; firstly, whole-brain white matter was studied and secondly, specific white matter tracts were isolated and analysed.

At the whole-brain level, numerous studies have identified abnormalities of white matter micro-structure in ASD but despite this, there is no clear picture of the underlying neural pathology. This may in part be due to differing protocols for acquisition and processing of diffusion MRI data. Therefore for whole-brain white matter examination, this study aimed to analyse an optimally pre-processed, high quality DTI dataset to investigate whether there were true differences in white matter organisation between participants with ASD and controls. Results from this study indicated that relative to controls, the ASD group showed significant disruption of white matter microstructural organization in regions of seven major association, projection and commissural tracts. This abnormal white matter was characterised by reduced FA and AD and increased MD and RD. A typical explanation of these findings would be that white matter "integrity" is reduced in ASD, however recent research has revealed that the interpretation of tensor derived metrics such as FA is likely to be highly ambiguous (Jeurissen *et al.*, 2012). It has been suggested that isolation of complete white matter tracts using diffusion tractography is likely to provide more readily interpretable information about "structural connectivity" in ASD (Jeurissen *et al.*, 2012).

A number of diffusion tractography studies have been reported in the ASD literature, however the majority of these have used a diffusion tensor tractography (DTT) approach. Recent research has suggested that there are limitations of the DTT approach that are likely to have profound implications for the interpretation of results from any study using this methodology

(Jeurissen *et al.*, 2012). An alternative tractography method based on constrained spherical deconvolution (CSD) (Tournier *et al.*, 2007) avoids a number of critical confounds that are associated with DTT. This study used CSD-based tractography in order to comprehensively assess white matter organisation in specific tracts that are important in visuospatial processing; the inferior fronto-occipital fasiculus (IFOF), arcuate fasiculus and forceps major and minor. To our knowledge, this is the first time that this advanced diffusion tractography method has been used in autism research. In addition, this study examined the relationships between the level of white matter organisation in the isolated tracts and two measures of behaviour - visuospatial processing performance and autism severity. Results revealed that there was disruption of white matter in inter- and intra-hemispheric white matter tracts in ASD, which was associated with both autism severity and visuospatial processing performance. Significant alteration of white matter organization was present in the right IFOF and the forceps major. Increased severity of stereotyped behaviours and restricted interests correlated with reduced integrity of white matter in the forceps major, and poorer visuospatial processing was associated with disrupted white matter in the right IFOF.

2.3. Integration of functional and structural connectivity data

Despite the extensive evidence for disrupted functional and structural connectivity in ASD, no previous studies have attempted to integrate these types of connectivity analysis. Multimodal (structure/function) connectivity studies in typically developing populations have shown that there are strong associations between white matter organisation, functional connectivity and behaviour. The ultimate aim of this study was to investigate, for the first time, how structural connectivity, functional connectivity and behaviour are related in ASD. To do this, functional connectivity data were used to define seed regions for diffusion tractography analysis. This approach resulted in isolation of white matter tracts that directly linked pairs of brain regions that showed abnormal functional connectivity. Microstructural organization of these white matter tracts was assessed and correlated with both functional connectivity and behavioural measures to provide a comprehensive

examination of the relationships between brain structural connectivity, functional connectivity and behaviour in ASD. Analysis of white matter in these tracts revealed that microstructural organisation was disrupted in some tracts that linked regions showing abnormal functional connectivity. In addition, there were strong correlations between measures of white matter microstructure, functional connectivity and behaviour, which provide a fascinating insight into the relationships between brain structure, brain function and information processing in both neurotypical controls and individuals with ASD. This novel multimodal imaging study reports, for the first time, direct associations between abnormal functional connectivity and disrupted white matter structure in ASD. It reveals that there are significant correlations between the microstructural integrity of white matter, functional connectivity, visuospatial processing speed and autism severity.

3. Implications

Overall, this study adds considerably to the knowledge of how brain structure, function and behaviour are related in ASD. Results of the functional connectivity analysis during mental rotation offer an insight into the neural underpinnings of atypical visuospatial processing in ASD. A novel tractography method has been used for the first time in ASD and reveals microstructural abnormalities in major association and inter-hemispheric tracts. Again for the first time in ASD, a multimodal connectivity study has been performed, which allows direct investigation of the impact of structural pathology on functional connectivity and behaviour. These results make a significant contribution to our understanding of the pathophysiology of neural connectivity in ASD.

It is crucial to understand the complex neural pathophysiology of autism that could lead to the development of therapeutic interventions that could plausibly improve white matter organization and increase inter-regional connectivity, in turn diminishing the core deficits that define ASD.

4. Limitations

Limitations to this work are discussed separately in each chapter in detail. The primary limitation of this work is the study population. Participants with

ASD were limited to male, right-handed individuals with average or above average IQ. Therefore results are very specific to this group and are not representative for all individuals on the spectrum. Participants ranged in age from 13 to 21 years, a time period in which there are significant neurodevelopmental changes. The ASD and control participants were very well matched on age and IQ, to minimise the potential impact of development and intelligence on the neuroimaging data. The results reported from functional connectivity MRI and CSD tractography are novel and require replication. Finally, a parametric measure of autism severity that could be related back to the neuroimaging data was lacking in this study. Response times during visuospatial processing were however extremely useful in interrogating how specific functional and structural abnormalities in ASD impacted on behaviour.

5. Future work

5.1. Attention Orienting study

I have obtained additional funding to continue neuroimaging research in ASD and am currently supervising a PhD student who is undertaking analysis of functional MRI data that was obtained from the participants in this study whilst they performed an attention orienting task. This attention orienting study will ultimately involve integration of functional and structural neuroimaging data using a similar approach to that described in this thesis.

Spatial orienting of attention is a cognitive function that allows a person to move their attentional focus from one location to another location in response to a stimulus. Attention orienting is of particular interest in ASD as orienting attention to new and important sources of information is fundamental to learning and normal socioemotional development (Dawson, 1991). Impaired attention orienting may be an important factor contributing to core social communication deficits in ASD (Dawson *et al.*, 2004; Greenaway and Plaisted, 2005; Zwaigenbaum *et al.*, 2005; Keehn and Joseph, 2008). The neural network subserving attention orienting is well established and involves interaction between the 'Dorsal Attention Network' (DAN) and the 'Ventral Attention Network' (VAN) (Corbetta and Shulman,

2002). Voluntary, goal-driven attention orienting appears to be controlled by the DAN. The VAN is involved in the control of stimulus-driven, automatic attention orienting, which is necessary for reorienting attention towards salient unexpected events. Preliminary analysis of the functional MRI data during this attention orienting task has revealed that relative to controls, the ASD group showed significantly more activation of the VAN during attention orienting. This finding is thought to reflect a compensatory increase in activation, which allowed the ASD group to perform behaviourally as well as controls during the task.

Recruitment to this study is ongoing and the aim is to involve another 25 individuals with ASD and 25 age- and IQ- matched controls. All individuals will undergo DTI scanning using the HARDI protocol outlined in chapter 4, which results in high quality diffusion data. It will be particularly interesting to interrogate structural organisation of the superior longitudinal fasciculus (SLF), a major white matter tract that plays an important role in attention orienting. It has recently been demonstrated that fibre-tracking algorithms that rely on the diffusion tensor will produce unreliable results in regions containing multiple fibre orientations. This may result in a failure to identify real white matter connections – for example where tracking terminates prematurely (Behrens *et al.*, 2007; Jeurissen *et al.*, 2011). The SLF passes through regions containing multiple fibre orientations, but no previous research in ASD has isolated the entire tract using a CSD-based tractography approach. In addition, parent- and self-reports will be obtained of the Social Responsiveness Scale (Constantino *et al.*, 2003), to provide quantitative data on the severity of autism symtoms.

5.2. Resting state connectivity

In neurotypical populations, a number of studies have investigated links between resting state functional connectivity and structural connectivity. Resting-state functional connectivity analysis is of great interest in ASD research, and multiple sites have collected resting state data on individuals with ASD. Recently an international consortium - the Autism Brain Imaging Data Exchange (ABIDE) - has been set up to share resting state and morphometric data for autism research. Our neuroimaging group in Trinity

College Department of Psychiatry is already actively involved in this consortium, and my PhD student will be collecting high quality resting state data on all new recruits to the project and submitting this to ABIDE. Integration of large-scale resting state functional connectivity datasets with morphological data will be exciting, however it will not allow for the interrogation of how white matter microstructural organisation impacts on functional connectivity in ASD. One of the challenges for pooling diffusion MRI data is that acquisition protocols (and scanners) vary widely. Experts in analysis of diffusion MRI have suggested that it is possible to pool such data, and over the next years, I will work towards arranging international pooling of diffusion MRI data, perhaps in association with the ABIDE consortium. The datasets from this would allow a very large integrative study of functional and structural connectivity in ASD.

5.3. Linking neuroimaging and genetics research

Future research will also focus on linking neuroimaging and genetics research. Although autism and ASDs are highly heritable, the genetics are complex and multiple susceptibility genes have been reported in the literature. Converging findings from neuroimaging and genetics research suggest that autism and ASDs are disorders of neural connectivity. It is hoped that the recent advances in functional and structural neuroimaging techniques will lead to the generation of robust neuroimaging endophenotypes (measurable traits that are both heritable and related to a specific aspect of the condition under investigation) that will aid genetics research. A recent study reported a significant association between a functional connectivity endophenotype in the human brain and variation in contactin-associated protein-like 2 (CNTNAP2) a member of the neurexin gene superfamily that has been associated with autism (Scott-Van Zeeland *et al.*, 2010). This study provides evidence to suggest that genetic variation at CNTNAP2 predisposes to disorders such as autism in part through modulation of frontal lobe connectivity.

Identifying robust neuroimaging endophenotypes will be a key goal of my future research. Functional and structural neuroimaging findings from studies to date have in general been very variable however. With the

advances in diffusion MRI analysis allowing for rigorous examination of structural connectivity and the collation of large datasets of resting state functional MRI data, I would hope that a clear neural pathology of autism and ASDs will emerge. Neuroimaging endophenotypes generated during ongoing structural and functional connectivity research in our autism research group will be used to examine the impact of functional genetic polymorphisms that have been implicated in the aetiology of autism and ASDs. For example, functional and structural connectivity endophenotypes will be invaluable in investigating the functional effects of risk variants of genes such as RELN or Integrin alpha 4, genes that are known to play a role in neural connectivity and that have been identified as susceptibility genes for autism.

5.4. Therapeutic interventions in ASD

The discovery that white matter structure can be influenced by experience is very exciting for autism research (Keller and Just, 2009; Scholz *et al.*, 2009). White matter structure is abnormal in numerous regions in autism but it may be possible to introduce therapeutic training to stimulate improvement in white matter organization. It is plausible that future treatments for autism will be developed from interventions that improve white matter organization and inter-regional brain connectivity. Such treatments may reduce the core deficits of ASD. I would hope to develop trials investigating the efficacy of training interventions that show potential to increase neural connectivity to see how they may benefit individuals with autism and ASDs.

6. Conclusion

There is extensive converging evidence from genetics, fMRI and DTI research that suggests that abnormal neural connectivity may underpin the core features of ASD. There is, however, a striking lack of research attempting to relate functional and structural connectivity, an approach that is crucial to increase understanding of the pathophysiology of aberrant connectivity in ASD. The central aim of this thesis was to investigate how structural connectivity and functional connectivity are related in ASD. To achieve this, a multi-modal functional MRI and DTI study was performed.

The study has provided novel insights into the neural correlates of atypical visuospatial processing in ASD, used advanced analytic techniques for the first time in autism research to reveal microstructural pathology of white matter and has found significant relationships between white matter structural pathology, functional connectivity and behaviour.

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