Implications of Cortical Structure and Connectivity in Autism Spectrum Disorder

Jacqueline Fitzgerald

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Doctor in Philosophy

Supervisors

Prof. Louise Gallagher

Dr. Jane McGrath

Declaration

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Summary

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by early-onset deficits in social communication and interaction together with recurrent restricted repetitive behaviours (APA, 2013). Insights from genetic, neuropathological and neuroimaging studies has led to the emergence of the 'altered cortical connectivity' theory of ASD (Casanova and Trippe, 2009, Courchesne and Pierce, 2005a, Just et al., 2004, Kana et al., 2007, Gilman et al., 2011) which suggests that the social, cognitive and behavioural deficits characteristic of ASD are underpinned by disrupted connectivity between distinct neural regions.

The aim of this thesis was to investigate functional connectivity of the dorsal and ventral attention networks during attention orienting, to assess structural connectivity of the superior longitudinal fasciculus (SLF) using constrained spherical deconvolution (CSD) based tractography, to explore differences in whole-brain structural connectivity of high angular resolution diffusion imaging data and finally, to evaluate the derivatives of cortical volume, cortical thickness and surface area in addition to their related measures, gyrification and sulcal depth, in a well-defined sample of ASD individuals and age and IQ matched neurotypical controls.

In Chapter 3, analyses found that despite similar behavioural performance, there were significant between-group differences in functional connectivity. In the dorsal attention network (DAN), the ASD group showed significantly weaker functional connectivity between ROIs and connected regions. In the ventral attention network (VAN), the ASD group showed stronger positive functional connectivity relative to the controls. The weaker functional connectivity shown by the ASD group in the DAN suggests weaker coherence between these brain areas while the strong positive

functional connectivity exhibited by the ASD group in the VAN suggests that individuals with ASD may generate compensatory mechanisms to achieve neurotypical behaviour.

In Chapter 4, investigation of the SLF found that white matter organisation is disrupted in the left SLF I and the right SLF II in ASD characterised by greater structural connectivity. Exploratory tract segmentation analyses illustrated that differences in white matter microstructure were driven by increased fractional anisotropy (FA), increased axial diffusivity (AD) and in some segments, reduced radial diffusivity (RD).

In Chapter 5, a whole-brain analysis of white matter organisation revealed reduced structural connectivity characterised by reduced FA in several fiber pathways including the corpus callosum, anterior thalamic radiation, corona radiata, forceps major and minor, cingulum and the inferior longitudinal fasciculus. Exploratory correlation analyses indicated that disrupted white matter organisation was associated with social and communication deficits and restricted repetitive behaviours in ASD.

In Chapter 6, evaluation of cortical measures demonstrated that although individuals with ASD have comparable global estimates of volume, thickness and area to controls, they demonstrated reduced cortical thickness and greater surface area in frontal, parietal and temporal regions. Disrupted cortical folding was characterised by greater gyrification and reduced sulcal depth.

Results presented here support the disrupted cortical connectivity theory of ASD, characterised by both increased and decreased connectivity. The cortical abnormalities identified may reflect a global disruption to cortical development which results in aberrant connectivity. These discoveries contribute to understanding the involvement of neural abnormalities in ASD development.

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Statement of Work

All of the data in this thesis was collected for three PhD projects completed by Dr. Jane McGrath (co-supervisor of this PhD), Sonja Delmonte PhD and myself, Jacqueline Fitzgerald under the supervision of Prof. Louise Gallagher. Dr. McGrath recruited and collected data from 25 ASD and 25 control participants. Dr. McGrath also researched and designed the attention orienting task used for the functional connectivity analysis (Chapter 3). Individuals from Dr. McGrath's cohort were included in all of the following analyses (Chapters 3 - 6). Dr. Delmonte recruited and collected data from 28 ASD and 27 control participants. Data obtained by Dr. Delmonte was included in the grey matter cortical structure analysis (Chapter 6). I recruited and collected data from 26 ASD and 24 control participants. The data gathered by myself was included in both of the structural connectivity analyses and the grey matter cortical structure analysis (Chapters 4 – 6). All the analyses in this thesis were performed by myself.

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1 General Introduction

1.1 Overview

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by early-onset deficits in social communication and interaction together with recurrent restricted repetitive behaviours (APA, 2013). The disorder was first reported by Leo Kanner (Kanner, 1943) and Hans Asperger (Asperger, 1944) when they independently described children with social idiosyncrasies. Kanner found that children were 'happiest when left alone' and appeared 'indifferent to visiting relatives' (Kanner, 1943). Asperger also noted that children were 'never able to become integrated into a group of playing children' (Asperger, 1944). Kanner and Asperger also documented unusual behavioural features such as 'making stereotyped movements with his fingers' (Kanner, 1943) and 'certain stereotypic movements and habits' (Asperger, 1944). They also noted that the children's language production deviated from the norm and reported 'muteness' or that the children's talk was 'like an adult' (Asperger, 1944) and that words appeared to have a 'specific, literal, inflexible meaning' (Kanner, 1943). These accounts of inappropriate or lack of social interaction, insistence on sameness and atypical use of language have been developed and modernized by a number of prominent researchers over the decades [see (Volkmar and McPartland, 2014) for review] and led to the recognition of 'infantile autism' as a disorder in the DSM-III under the category of pervasive development disorders (PDD) (APA, 1980). In the subsequent versions of the DSM, autism was reviewed and reclassified. Infantile autism was renamed 'autistic disorder' in the DSM-III-R and sixteen criteria for

diagnosis were categorised under three sub-headings; qualitative impairment in social interaction, communication and restricted interests.

1.2 Diagnosis

The introduction of the DSM-V has led to significant changes in diagnostic criteria for ASD (DSM-V; APA, 2013). Previously, in the DSM-IV-TR, autism, Asperger's syndrome, Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS), Rett's Syndrome and Childhood Disintegrative Disorder were included in the section on Pervasive Developmental Disorders (APA, 2000). In the DSM-IV-TR, diagnosis of autism was dependent on the presence of deficits in three domains; social interaction, communication and repetitive behaviours. The categorical diagnoses of autism, Asperger's Syndrome, and PDD-NOS were replaced with the umbrella term Autism Spectrum Disorder (ASD). In the DSM-V, a dyadic construct rather than a triadic construct was adopted for diagnosis. Social and communication symptom domains were merged into the 'social and communication deficits' (SCD) domain while restricted behaviours is now referred to as 'restricted and repetitive behaviours, interests or activities' (RRB). This development resulted from the observation that SCD and RRB were separable and highly heritable (Boomsma et al., 2008, Frazier et al., 2008). In addition, a rating system is used to determine the severity of symptoms; from level 1 'requiring support' to level 3 'requiring very substantial support'. To receive a diagnosis of ASD, deficits must be observed in both domains in multiple contexts, must have presented during the early developmental period, must cause clinically significant impairment of functioning and cannot be explained by intellectual disability or global developmental delay. For individuals without deficits in the restricted repetitive

behaviour domain, a new diagnosis of Social Communication Disorder was developed (APA, 2013).

1.3 Epidemiology

Large-scale population-based studies have estimated that the prevalence of ASD is approximately 1 – 2% of the population (Mattila et al., 2011, Kim et al., 2011, Idring et al., 2012, Saemundsen et al., 2013). Research suggests that ASD is 4 – 5 times more common in males than females however, the disparity appears to decrease in individuals with a co-morbid intellectual disability (Fombonne et al., 2011). In the United States, a report by the Center for Disease Control and Prevention (CDC) demonstrated that the rates of ASD have risen markedly over the last decade, signified by a 78% increase since 2007 (CDC, 2012). While this surge may be indicative of a genuine rise in ASD prevalence, it is also likely to reflect a greater awareness of the disorder as well as improved identification and diagnostic classification within the community. With the new approach for ASD diagnosis in the DSM-V, the impact on prevalence rates has yet to be determined. Some research has suggested that prevalence rates may fall by as much as 39.5% (McPartland et al., 2012). Others suggest it may fall by approximately 10% (Maenner et al., 2014) but this drop of 10% may simply reflect individuals who receive the new diagnosis of social communication disorder (Kim et al., 2014).

Over 70% of individuals with ASD have a co-morbid developmental, psychiatric or medical condition [see (Lai et al., 2014) for review] with intellectual disability and anxiety, in particular social anxiety disorder, occurring in almost half of the ASD population. Other developmental co-morbidities include attention-deficit hyperactivity

disorder, language difficulties and tic disorders. Psychiatric co-morbidities include depression, obsessive-compulsive disorder, eating disorders, oppositional defiant disorder and psychosis. Co-occurring medial diagnoses include epilepsy, immune dysregulation and gastrointestinal conditions such as constipation, inflammatory bowel disease and Crohn's disease [see (Lai et al., 2014) for review]. An increased number of co-morbidities have been associated with greater symptom severity in ASD (Mattila et al., 2010).

1.4 Aetiology

Extensive research within a number of scientific fields including genetics, neuropathology, neuroimaging in addition to environmental observations has lead to a greater understanding of the complex pathophysiological mechanisms involved in ASD. Evidence from each of these areas is discussed in more detail in the following paragraphs.

1.4.1 Genetic Factors

Comprehensive research literature suggests that there is a substantial genetic component in the aetiology of ASD. A review of 30 twin studies of ASD have reported high concordance rates in monozygotic twins of approximately 80 – 90% while dizygotic twins demonstrate concordances of under 10% (Ronald and Hoekstra, 2011). Genetic studies have demonstrated that ASD is a complex and heterogeneous disorder. Single gene disorders such as Fragile X, Rett or Angelman syndrome, cytogenetic lesions and rare de novo mutations account for only 10 – 20% of ASD diagnoses (Abrahams and Geschwind, 2008). No single rare risk variant has been able

to account for more than approximately 1% of diagnosed individuals. Genetic analyses, in particular genome-wide association study (GWAS) analyses, have identified a number of genes exhibiting common variation, which confer risk for ASD (Anney et al., 2010, Ma et al., 2009, Wang et al., 2009, Weiss et al., 2009, Abrahams and Geschwind, 2008). Thus it has been proposed that a significant number of common risk variants, which have a small effect size and/or the presence of rare variants that have a large effect size may contribute to the phenotypic expression of ASD (State and Levitt, 2011, Marshall and Scherer, 2012).

Genetic ASD studies have implicated a number genes involved in neurodevelopment, including neuroligin 3 and 4 (NLGN3 and NLGN4), neurexin 1 (NRXN1), contactin associated protein-like 2 (CNTNAP2), MET and SH1, 2 and 3 multiple ankryin repeat domains (SHANK1, SHANK2, SHANK3) [see (Won et al., 2013) for review]. Pathway and network based analyses of common genetic variation implicate processes such as synaptogenesis, among others, which subsequently impair synaptic functioning. Neurexin and neuroligin are synaptic cell adhesion molecules concentrated in pre and post-synaptic membranes that regulate excitatory and inhibitory synaptic development and function. Mutations in these genes are thought to contribute to intellectual disability (Blundell et al., 2010) and impaired social functioning (Jamain et al., 2008). CNTNAP2, a member of the neurexin family, is a neuronal transmembrane protein, which regulates neuron-glia interactions and has been linked with language impairment (Whalley et al., 2011), altered functional connectivity (Scott-Van Zeeland et al., 2010) and disrupted structural connectivity (Clemm von Hohenberg et al., 2013, Dennis et al., 2011) in ASD. The MET gene encodes a tyrosine kinase receptor that promotes neuronal growth and cell migration

and SHANK genes encode scaffolding proteins in the post-synaptic membrane. In ASD, these molecules are also thought to negatively influence social functioning (Won et al., 2012, Levitt and Campbell, 2009) and cognitive ability (Wang et al., 2011). These genes in addition to others are thought to operate in a large functional network which influences the formation and function of synapses. This suggests that disrupted synaptic and neuronal connectivity may contribute to the aetiology of ASD (Gilman et al., 2011).

Furthermore, a number of *de novo* (sporadic rather than inherited) Copy Number Variant (CNV) risk loci have been identified in ASD. CNV is a form of structural variation in which multiple nucleotide bases, the building blocks of DNA, in the genome are altered. Two comprehensive GWAS studies of over 2,000 individuals with ASD identified polymorphisms at multiple loci; 16p11.2, 22q.11.2, 1q21.1 and 15q13.3 (Sanders et al., 2011, Pinto et al., 2010). These studies found that the proportion of de novo CNVs was 3 – 5 times greater in ASD families relative to controls and Pinto et al. (2010) established that individuals with more than one *de novo* mutation presented with a more severe clinical profile. It has been suggested that sporadic mutations are more common in 'singleton' families, families with two or more children but only one diagnosed with ASD (Michaelson et al., 2012, Neale et al., 2012). Therefore these mutations are likely to be exerting moderate effects. Conversely, the majority of ASD risk at the genetic level is liable to be conferred by multiple common variations (Abrahams and Geschwind, 2008). Notably, some of these genetic variants have been associated with other neuropsychiatric disorders. Research has shown that NRXN1 deletions and CNTNAP2 polymorphisms are linked with schizophrenia (Levinson et al., 2012, Kirov et al., 2009, Friedman et al., 2008) and deletions and duplications at

16p11.2 were also found to be associated with psychosis (Levinson et al., 2012, McCarthy et al., 2009) and bipolar disorder (Georgieva et al., 2014). Identification and analysis of traits common to both ASD and these disorders may be helpful in elucidating the role of genetic variation in ASD pathophysiology.

1.4.2 Environmental Factors

Studies investigating the role of environmental factors in ASD aetiology have probed the impact of external non-heritable factors during preconception, prenatal, birth and post-natal time periods. Advanced parental age and parental immigration were identified as risk factors for ASD (Magnusson et al., 2012, Gardener et al., 2009, Bolton et al., 2014). Furthermore, prolonged exposure to chemical toxins prior to conception was also considered to be a risk, however these studies were retrospective and susceptible to bias [see (Rossignol et al., 2014) for review]. A number of pre-natal risk factors have also been reported. Pre-natal exposure to the antiepileptic medication Valproate has been associated with a 7-fold increase in ASD development as well as a number of neurodevelopmental disorders including ADHD and dyspraxia (Bromley et al., 2013). Ingestion of tricyclic antidepressants such as selective serotonin reuptake inhibitors (SSRIs) and non-selective monoamine reuptake inhibitors during pregnancy has also been considered contributory factors (Rai et al., 2013). Prenatal exposure to SSRIs was found to confer a 2-fold increased risk of ASD (Gidaya et al., 2014, Croen et al., 2011). Gestational exposures to toxicants including pesticides, solvents and air pollutants have also been implicated [see (Rossignol et al., 2014) for review]. Birth complications including pre- and post-term birth, hypoxia, fetal distress, Caesarean section and prolonged labour are believed to confer risk for pervasive

developmental disorders including autism (Guinchat et al., 2012, Gardener et al., 2009). A history of ante- and post-natal maternal depression was linked with an estimated 60% increase in risk of ASD development (Rai et al., 2013). Although the mechanism for this link has not yet been elucidated, it has been postulated that it may be due to in utero exposure to serotonergic agents which are involved in neurogenesis, neural apoptosis and synaptogenesis (Rai et al., 2013). However, it must be noted that a number of these risk factors have been implicated in a variety of disorders such as ADHD (Bromley et al., 2013, Clements et al., 2014) and dyspraxia (Bromley et al., 2013, Kipiani et al., 2007), therefore are not specific to ASD.

1.4.3 Neuropathology

Neuropathological studies on autism have reported some consistent findings [see (Zikopoulos and Barbas, 2013) for review]. Atypical neuronal quantity and size including Purkinje cells (GABAergic neurons) and pyramidal neurons have been implicated in the pathophysiology of ASD. Children with ASD presented with a 58% and 67% increase in neurons in the fronto-insular cortex and the prefrontal cortex respectively relative to controls (Courchesne et al., 2011, Santos et al., 2011). Conversely, a 10 – 20% reduction in the number of neurons was observed in the brains of children and adults with ASD in the amygdala, lateral nucleus, fusiform gyrus and visual cortex (Schumann and Amaral, 2006, van Kooten et al., 2008). However, another study of 9 ASD adult brains aged between 30 and 44 showed no difference in neuronal size or density (Zikopoulos and Barbas, 2010). A reduced number of Purkinje cells in the cerebellum have been identified in studies of both children and adults [see (Gadad et al., 2013, Palmen et al., 2004) for reviews]. Furthermore, a reduction the

synthesizing enzyme for GABA was also found in the dentate gyrus neurons, which communicate with Purkinje cells in adults (Yip et al., 2009). Although neuronal cell numbers were comparable, pyramidal neurons were found to be smaller in the dorsolateral prefrontal cortex (Jacot-Descombes et al., 2012). Some studies have suggested that these findings are due to reduced levels of signalling proteins involved in neuronal migration and apoptosis (Fatemi and Halt, 2001, Fatemi et al., 2001b, Fatemi et al., 2001a, Araghi-Niknam and Fatemi, 2003).

Zikopoulos and Barbas (2010) provided support for an axonal pathology, specifically in the prefrontal cortex in ASD. A reduced number of axons connecting regions over long distances below the anterior cingulate and a decreased number of axons connecting the adjacent orbitofrontal cortex was reported. Reduced myelination was also observed in axons connecting the anterior cingulate and orbitofrontal cortices (Zikopoulos and Barbas, 2010). Greater density of pyramdial dendritic spines, which faciliate communication between axons, have also been implicated in ASD (Hutsler and Zhang, 2010).

Irregular glial cell activation has also been associated with the development of ASD. Significant activation of microglia, astroglia as well as production of neuroglia derived cytokines was demonstrated in post-mortem tissue of 11 autistic brains (Vargas et al., 2005). Increased glial density as well as increased microglial activation has also been reported in the dorsolateral prefrontal cortex (Morgan et al., 2010). Glial cells primarily function to provide structural support to the neuronal network, produce myelin and enhance neurotransmission and are also believed to play a role in neuronal migration, function and formation of the minicolumn and apoptosis (Marin-Teva et al., 2004).

Post-mortem studies have also supported a role for minicolumns in the pathology of ASD. A minicolumn is a radially oriented network composed of pyramidal projection neurons and fibers, which provides a basic architectural structure within the neo-cortex (Casanova et al., 2002b, Casanova et al., 2002a). Examination of minicolumn morphometry revealed an increased number of narrowed minicolumns (Buxhoeveden et al., 2006, Casanova et al., 2002b, Casanova et al., 2002c, Casanova et al., 2010, Casanova et al., 2006), a reduced distance between columns (Casanova et al., 2006), a reduction in the number of cells per columns (Casanova et al., 2002b, Casanova et al., 2002c) and/or a reduced neuronal cell size (Casanova et al., 2006, Casanova et al., 2013) in ASD brains, particularly in frontal regions. The greater number of minicolumns reported is thought to reflect the early brain overgrowth characterised in ASD (Redcay and Courchesne, 2005) while the reduced distance between these columns has been linked to an imbalance in the maturation of excitatory and inhibitory cells during cortical development (Casanova et al., 2013). It has been suggested that the reduction in neuronal cell size, specifically the cell soma and nucleus may represent a reduced capacity for long-range communication due to diminished metabolic activity thus creating a bias for short-range connectivity in ASD (Opris and Casanova, 2014).

1.4.4 Atypical Neural Development

Evidence from both a neuropathological and neuroimaging perspective suggests that ASD is characterised by early brain over-growth followed by premature slowing of neural growth (Carper and Courchesne, 2005, Aylward et al., 2002, Courchesne, 2004) possibly owing to the abnormal synaptic pruning, dendritic arborisation and

myelination which occur throughout the lifespan (Schumann and Nordahl, 2011, Nickl-Jockschat and Michel, 2011). During typical growth, it is believed that these processes function to develop the co-ordination of neural networks by eliminating ineffective neural connections. Thus, researchers have postulated that disruptions to cellular mechanisms such as apoptosis, synaptic and axonal pruning, neurogenesis and synaptogenesis contribute to aberrant neural development in ASD (Piven et al., 1996, Frith, 2003, Geschwind and Levitt, 2007).

In neurotypical individuals, it has been established that grey and white matter developmental trajectories differ across the lifespan [see (Mills and Tamnes, 2014) for review], however there has been some discrepancy regarding the period at which tissue acceleration and/or deceleration occurs. Some studies suggest that peak grey matter volume occurs in early childhood and begins to decline in adolescence before reaching a plateau during adulthood (Wierenga et al., 2014, Lebel and Beaulieu, 2011, Tamnes et al., 2013, Aubert-Broche et al., 2013) while other studies have indicated that grey matter follows an inverted U-shaped trajectory with initial peak grey matter volumes occurring in late childhood or early adolescence (Raznahan et al., 2011). Reports indicated that white matter expansion occurs across childhood into adolescence before stabilising (Aubert-Broche et al., 2013, Lebel and Beaulieu, 2011) or declining (Kochunov et al., 2012) into adulthood however the age at which white matter acceleration occurs and ceases has varied across these studies.

In ASD, several analyses have revealed abnormal developmental trajectories of both grey and white matter tissue in studies that have compared ASD groups with neurotypical populations. Age-related abnormalities in grey-matter development have been reported in a number of studies. Cross-sectional analyses identified age-related

differences in grey matter volume in the striatum, amygdala, temporoparietal junction, precentral gyrus and middle cingulate cortex in individuals with ASD relative to controls (Langen et al., 2009, Greimel et al., 2013) however, the orientation of these differences were region specific. In a cohort ranging from 8 – 50 years, Greimel et al. (2013) found greater grey matter volumes in the amygdala, temporoparietal junction and middle cingulate gyrus from childhood into middle adulthood in ASD relative to controls. In this same study, reduced grey matter from mid to late adulthood was observed in these regions reflecting a U shaped trajectory. Conversely, the precentral gyrus revealed an opposing pattern of development whereby grey matter appeared to follow an inverted U shaped trajectory (Greimel et al., 2013). Similar discrepancies were notable within the striatum of 99 ASD participants aged 6 – 25 years (Langen et al., 2009). Both the caudate nucleus and the nucleus accumbens showed reduced grey matter during childhood but greater levels in adolescence in ASD in comparison with controls. However, there was an increase in volume in the putamen throughout childhood and adolescence with comparable levels in adulthood relative to controls (Langen et al., 2009). Collectively, these results illustrate that the developmental trajectory of grey matter is abnormal in ASD and although the pattern of abnormality has yet to be elucidated, it appears to be region specific.

Age-related anomalies in white matter development have also been reported in a number of ASD studies (Hua et al., 2013, Kleinhans et al., 2012, Mengotti et al., 2011). A longitudinal study of 13 ASD adolescent males scanned approximately 3 years apart revealed decelerated white matter development in parietal, temporal and occipital regions relative to the rapid whole brain white matter growth demonstrated by 7 typically developing individuals (Hua et al., 2013). This global age-related

divergence was also apparent in a cohort of 20 ASD children aged 4 – 14 years (Mengotti et al., 2011). In an ASD population ranging from 13 – 35 years of age, Kleinhans et al. (2012) illustrated that reduced white matter is apparent during childhood into adolescence in a number of association, commissural and projection fiber pathways but this reduction appears to normalise over time and reach equivalent volumes during the mid-twenties. As with grey matter, these studies show that there is abnormal white matter development in ASD. This may be due to a slowing of typical white matter development and/or an accelerated reduction of excess white matter tissue present from early brain overgrowth. No clear pattern of abnormality has been established thus requires further investigation.

1.5 Neuroimaging

Scientific advances in magnetic resonance imaging (MRI) have enabled researchers to investigate brain structure and function *in vivo* non-invasively in a number of neurodevelopmental disorders including autism, ADHD, Fragile X, velocardiofacial syndrome, Williams syndrome and Turner syndrome [see (Dennis and Thompson, 2013) for review]. Initially, research focused on investigating brain structure and function of individual regions however, it has become apparent over the past two decades that the brain requires a series of neural networks to achieve successful cognitive functioning. A review of brain structure, brain function and functional and structural connectivity in ASD is presented in the following section.

1.5.1 Brain Structure in ASD

Neuroimaging methods have been used to investigate structural abnormalities in ASD. Results yielded from these studies have been varied [see (Amaral et al., 2008, Anagnostou and Taylor, 2011) for reviews]. Meta-analyses of grey matter volumetric studies have attempted to identify regions which demonstrate consistent aberrant cortical structure in ASD. Studies employing volume based morphometry (VBM), a method used to investigate localised or voxel-wise differences in neural anatomy, have reported white matter anomalies in ASD relative to controls. Additionally, it has been hypothesised that grey and white matter abnormalities may be linked or co-occurring in brain regions. With the advancements in technology, modernised approaches such as evaluating cortical thickness and surface area have been adopted to further elucidate structural abnormalities in ASD. The findings from these studies are reported in the following paragraphs.

1.5.1.1 Grey Matter Volumetric Studies

Evidence from two meta-analytic studies demonstrated an increase in grey matter volume in both the cerebellum and the caudate (Cauda et al., 2011, Stanfield et al., 2008). Cauda et al. (2011) also identified grey matter volume increases in the anterior and posterior cingulate, insula, precuneus and the middle temporal and fusiform gyri in addition to grey matter volume decreases in cerebellar tonsils, inferior parietal lobule, amygdala, insula, precuneus and middle temporal and precentral gyri.

1.5.1.2 White Matter Volumetric Studies

Volume based morphology (VBM) analyses have also identified white matter irregularities in ASD, particularly in terms of the volume and size of the corpus

callosum (Alexander et al., 2007a, Keller et al., 2007, Shukla et al., 2010, Bloemen et al., 2010, Noriuchi et al., 2010). Widespread compromise in both white matter volume and structure has also been identified in frontal (Ke et al., 2009, Noriuchi et al., 2010, Barnea-Goraly et al., 2004, Sundaram et al., 2008, Bloemen et al., 2010, Cheung et al., 2009), temporal (Noriuchi et al., 2010, Barnea-Goraly et al., 2004, Bloemen et al., 2010, Cheung et al., 2009), parietal (Ke et al., 2009, Bloemen et al., 2010) and cingulate (Noriuchi et al., 2010, Barnea-Goraly et al., 2004, Ke et al., 2009, Bloemen et al., 2010) regions. Another study revealed no difference in cortical grey or white matter volume in ASD relative to controls (Riedel et al., 2014). Researchers postulated that age, gender, cognitive ability, co-morbidity and methodological differences may have influenced study outcomes (Cauda et al., 2011, Amaral et al., 2008, Stanfield et al., 2008).

1.5.1.3 Concordant Abnormalities in Grey and White Matter

A study published recently aimed to elucidate if white and grey matter abnormalities were concordant across VBM and diffusion tensor imaging (DTI) studies (Cauda et al., 2014). Diffusion tensor imaging is an effective tool used to investigate white matter microstructural organisation (see Chapter 2, Section 2.4.3 for further details). Evidence from this study suggests that a significant topological relationship exists between grey and white matter structural abnormalities where negative concordance (decrease in both tissues) was most commonly observed, particularly in the right hemisphere. This study also demonstrated a concordance between cortical regions and their white matter connections. The frontal and dorsal parietal tracts, primarily the superior longitudinal fasciculus (SLF) and the anterior corona radiata

revealed a negative concordance with the cortical structures they connect while the posterior corona radiata demonstrated a positive concordance (increase in both tissues) with the neural regions it connects (Cauda et al., 2014). This suggests that a relationship exists between grey and white matter structural anomalies in ASD thus indicating that abnormalities in both tissues jointly contribute to the disorder.

1.5.1.4 Advanced Methods for Studying Cortical Structure

While volumetric studies are useful in localising structural irregularities to specific regions in the brain, VBM studies cannot offer information regarding anomalies within the sub-components of cortical volume; cortical thickness and surface area. Thus over the past few years, the focus of grey matter studies has shifted towards the independent investigation of these cortical volume derivatives. These measures have been found to be genetically uncorrelated, phenotypically independent and highly heritable (Winkler et al., 2010, Panizzon et al., 2009, Sanabria-Diaz et al., 2010). As with volumetric studies, results have been inconsistent. Increased cortical thickness has been observed in some studies (Hyde et al., 2010, Ecker et al., 2013, Doyle-Thomas et al., 2013a, Libero et al., 2014) while others report cortical thinning in ASD (Misaki et al., 2012, Wallace et al., 2010, Chung et al., 2005, Ecker et al., 2014). Similarly, discrepant surface area findings have been described (Raznahan et al., 2010, Mak-Fan et al., 2012, Wallace et al., 2013, Raznahan et al., 2012, Doyle-Thomas et al., 2013c, Ecker et al., 2014, Hazlett et al., 2011, Libero et al., 2014, Ecker et al., 2013). Similar to volumetric analyses, age, IQ and different phenotypic profiles are likely to contribute to the variability of findings.

1.5.2 Brain Function in ASD

Functional magnetic resonance imaging (fMRI) methods have enabled researchers to investigate the neural underpinnings of ASD-related difficulties in social-communication and restricted repetitive behaviours [see (Pina-Camacho et al., 2012) for review]. Neural activation, measured by the blood-oxygen-level-dependent (BOLD) response (see Chapter 2, Section 2.4.2 for more details) during both cognitive tasks and at rest can be examined to establish brain regions that facilitate specific cognitive functions.

In relation to social communication, numerous studies have employed face processing, gaze-processing and mentalisation-related cognitive tasks to probe social and communication deficits in ASD (Kleinhans et al., 2008, Koshino et al., 2008, Monk et al., 2010, Ashwin et al., 2007, Kana et al., 2009, Mason et al., 2008, Schmitz et al., 2008, Pelphrey et al., 2005). Abnormal activation was consistently reported in the limbic system, fusiform gyrus, frontal lobe, inferior frontal gyrus, temporal lobe, anterior cingulate and the prefrontal cortex across these studies (Pina-Camacho et al., 2012, Nickl-Jockschat et al., 2014).

Fewer studies have evaluated the phenomena of frequent restricted repetitive behaviours in ASD. These behaviours have been linked with disrupted executive functioning. Therefore, executive function tasks incorporating response inhibition, attention, cognitive flexibility and spatial working memory have been used to evaluate insistence on sameness and obsessive traits in ASD (Agam et al., 2010, Kana et al., 2007, Monk et al., 2009, Schmitz et al., 2006, Shafritz et al., 2008, Thakkar et al., 2008, Haist et al., 2005, Ambrosino et al., 2014). Irregular activation of the cingulate, particularly the anterior cingulate and the frontal lobe has most commonly been

identified in ASD during performance of executive function tasks (Pina-Camacho et al., 2012). Research indicates that these regions operate in a series of neural networks in order to subserve higher cognitive functioning thus it holds that the impaired cognitive features in ASD may be attributed to compromised brain networks.

1.5.3 Brain Connectivity in ASD

Insights from genetic, neuropathological and neuroimaging studies has led to the emergence of the 'altered cortical connectivity' theory of ASD (Casanova and Trippe, 2009, Courchesne and Pierce, 2005a, Just et al., 2004, Kana et al., 2007, Gilman et al., 2011). This theory suggests that the social, cognitive and behavioural deficits characteristic of ASD are underpinned by disrupted connectivity between distinct neural components or regions. The term cortical connectivity includes both functional and structural connectivity. Functional connectivity refers to the temporal communication or co-ordination of processing between brain regions during a cognitive process or at rest. Structural connectivity refers to the physical connections between neuronal elements at the micro level and between brain regions at the macro level. Aberrant functional and structural connectivity have been reported in ASD, the details of which are outlined below.

1.5.3.1 Functional Connectivity in ASD

Functional connectivity MRI (fcMRI) analyses typically measure the synchronicity of brain regions by examining associations in brain activation over a period of time (Friston, 2011) and can be implemented to investigate neural interaction during cognitive processing. Disrupted functional connectivity has been described in several ASD studies which suggest that both long-range and short-range connectivity is

disturbed in the disorder [see (Vissers et al., 2012) for review]. Initial evidence supported a theory of long-range under-connectivity and short-range over-connectivity in ASD (Courchesne and Pierce, 2005b) however, current collated evidence implies that patterns of connection dysfunction are more variable and may be characterised by aberrant (under and over) functional connectivity across both long and short range fiber pathways (Muller et al., 2011, Kana et al., 2014b, Wass, 2011).

Aberrant functional connectivity has been demonstrated during several cognitive processes such as visuospatial processing (McGrath et al., 2012, Liu et al., 2011b, Damarla et al., 2010), cognitive control (Solomon et al., 2009, Solomon et al., 2013), response inhibition (Agam et al., 2010, Lee et al., 2009), face processing (Monk et al., 2010, Kleinhans et al., 2008, von dem Hagen et al., 2014, Swartz et al., 2013, Sato et al., 2012, Murphy et al., 2012), social reward processing (Delmonte et al., 2013), sentence and language comprehension and processing (Mizuno et al., 2011, Kana et al., 2006), mentalising (Kana et al., 2014a, Fishman et al., 2014, Kana et al., 2009, Mason et al., 2008) and working memory (Koshino et al., 2008, Just et al., 2007) as well as at rest, a period of time where the brain is not explicitly engaged in an active cognitive task (von dem Hagen et al., 2013, Weng et al., 2010, Di Martino et al., 2011, Cherkassky et al., 2006, Di Martino et al., 2013, Monk et al., 2009, Shih et al., 2010, Fishman et al., 2014, Redcay et al., 2013).

What constitutes long and short-range connectivity has been a source of disparity across fcMRI studies (Vissers et al., 2012) and may contribute to inconsistent findings in ASD. Therefore functional connectivity observations are explained here in terms of neural regions rather than by long and short-range connectivity. Reduced functional connectivity between fronto-parietal regions has been reported in ASD

populations during performance of several cognitive tasks (Damarla et al., 2010, Just et al., 2007, Just et al., 2004, Kana et al., 2006, Kana et al., 2009, Solomon et al., 2009) and during resting state (Cherkassky et al., 2006, Di Martino et al., 2013). Conversely, increased functional connectivity between these fronto-parietal regions during the resting state has been reported (Fishman et al., 2014, Redcay et al., 2013, Shih et al., 2010). Sub-cortical connectivity has also revealed a diversity of results. Sub-cortical connectivity refers to the functional projections within sub-cortical structures such as the hippocampus, amygdala and the basal ganglia or between these sub-cortical structures and the cortex. Increased functional connectivity was demonstrated between the striatum and frontal regions during ASD reward processing and at rest (Delmonte et al., 2013, Di Martino et al., 2013). Furthermore, greater functional coherence was observed in projections between the thalamus and the insula, postcentral gyrus and middle frontal regions (Mizuno et al., 2006). However, a decrease in functional connectivity was found between the thalamus and prefrontal, motor and parietal-occipital regions during rest in an ASD population (Nair et al., 2013) as well as between connections from the insula to the precuneus, middle and inferior frontal gyri and the inferior parietal lobule during response inhibition and language processing (Kana et al., 2007, Mizuno et al., 2011). Ebisch et al. (2011) specified that the insula displayed both over and under-connectivity patterns in autism when functionally connected to emotional processing regions, particularly the posterior cingulate and the amygdala. Functional connectivity analyses demonstrated increased co-ordination of processing between the amygdala and the ventral medial prefrontal cortex, posterior cingulate, superior temporal sulcus, insula and thalamus during face processing (Kleinhans et al., 2008, Monk et al., 2010, Murphy et al., 2012) but reduced

co-ordination with these regions during rest (von dem Hagen et al., 2014). Functional connectivity patterns involving the temporoparietal junction and the anterior cingulate are also variable and given their role in theory-of-mind processing and cognitive control respectively, these regions are of considerable interest in ASD research. Reduced functional connectivity has been reported between the anterior cingulate and fronto-parietal regions during visuospatial processing and response inhibition (Agam et al., 2010, Kana et al., 2007) however during resting state, Shih et al. (2010) noted increased functional connectivity between the anterior cingulate and fronto-parietal areas. Similarly, reduced functional connectivity was observed between the temporoparietal junction and medial prefrontal, motor and cingulate cortices during mentalising (Mason et al., 2008, Kana et al., 2014a) however increased functional connectivity was observed between these regions during rest (Fishman et al., 2014). Researchers have suggested that functional connectivity is state dependent (You et al., 2013). This study reported that synchronicity of brain regions reduced and became more localised during a sustained attention task relative to rest in typically developing individuals while conversely, functional connectivity increased and became more diffuse in ASD participants. You et al. suggests that over-connectivity may reflect a maladaptive compensatory mechanism in ASD while others have implied that it is may be beneficial to overcoming behavioural deficits (Greene et al., 2011). The divergent patterns of functional connectivity observed across fcMRI studies have compelled researchers to speculate that the cognitive state, specific network, methodological approach, severity and age of cohort have influenced interpretation of findings in ASD studies to date (Kana et al., 2014b).

1.5.3.2 Structural Connectivity in ASD

Anatomical or structural connectivity is predominantly measured *in vivo* using diffusion Magnetic Resonance Imaging (MRI). Diffusion MRI is an imaging method based on the measurement of water molecular diffusion and is highly sensitive to subtle changes in white matter microstructure (Jones and Leemans, 2011). The diffusion tensor model is the most common methodological approach used in analysis of diffusion MRI data as it enables determination of the orientation and coherence of white matter fiber pathways based on diffusion properties (Alexander et al., 2007b). If water movement is unrestricted by cellular barriers, diffusion will occur equally in all directions. This freely permitted diffusion is known as isotropic diffusion. Conversely, if the passage of water molecules is impeded by barriers such as cell membranes or myelin sheaths which are present throughout white matter tissue, diffusion becomes anisotropic i.e. does not occur equally in all directions (Beaulieu, 2002). Axonal myelin sheaths restrict water molecule diffusion in white matter thus driving water diffusion parallel to the axonal direction while impeding perpendicular diffusion. Greater anisotropy is observed in regions composed of abundant axonal fibers therefore, white matter tracts are distinguishable using DTI methods due to their highly anisotropic diffusion properties.

Fractional anisotropy (FA) is the most commonly used measure of white matter microstructural organisation and is reflective of changes in axonal density and diameter, myelination and fiber tract direction coherence (Beaulieu, 2002). Fractional anisotropy is considered to be a measure of structural connectivity i.e. higher FA values are considered to represent stronger structural connectivity between neural regions (Alexander et al., 2007b). Thus differences in this metric in clinical imaging

studies are thought to illustrate compromised structural connectivity. Although FA is sensitive to changes in white matter architecture, it cannot elucidate the specific type of change. Axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) metrics can be estimated to aid description of microstructural changes as different diffusion metrics appear to represent different white matter pathologies. Radial diffusivity is believed to represent the extent of myelination of axons while axial diffusivity is thought to reflect axonal integrity (Song et al., 2002, Song et al., 2005). Linear diffusion coefficient (CL) and planar diffusion coefficient (CP) are diffusion metrics which can incorporate geometric information of the diffusion tensor hence providing more interpretable information relating to abnormalities in white matter architecture (Westin et al., 2002). Linear diffusion coefficient is closely related to axial diffusivity while planar diffusion coefficient is closely related to radial diffusivity. Given that these metrics offer more representative information, they were used in addition to FA to investigate white matter organisation in the SLF. A high CL value indicates a singular dominant fiber orientation in a voxel while a high CP value suggests a number of crossing fibers in a voxel (Vos et al., 2012).

Three main methodological approaches have been implemented to evaluate white matter organisation in ASD, voxel-based morphometry (VBM) whole brain analysis, tract-based spatial statistics (TBSS) and region of interest (ROI) diffusion tensor imaging (DTI) tractography. Initially, VBM analysis was the primary tool used to investigate white matter architecture in ASD studies however, with the advancement of technology and knowledge of methodological difficulties with this method, the tract-based spatial statistics method was developed (Smith et al., 2006). More recently, ROI tractography allows for the reconstruction of white matter tracts based

on their dominant diffusion orientation thus providing tract-specific microstructural information not available via other methods. An overview of study outcomes using TBSS and ROI DTI-based tractography are described below.

1.5.3.2.1 Tract-Based Spatial Statistical Studies of ASD

A number of TBSS studies of white matter microstructure have been performed in ASD, the majority of which have reported widespread reduced FA across several brain regions. Reduced structural connectivity has been observed in the anterior thalamic radiation (Cheon et al., 2011, Barnea-Goraly et al., 2010, Bakhtiari et al., 2012, Shukla et al., 2011), corpus callosum (Cheon et al., 2011, Jou et al., 2011b, Barnea-Goraly et al., 2010, Bakhtiari et al., 2012, Shukla et al., 2011), uncinate fasciculus (Cheon et al., 2011, Jou et al., 2011b, Bakhtiari et al., 2012, Shukla et al., 2011), cingulum (Jou et al., 2011b, Barnea-Goraly et al., 2010, Bakhtiari et al., 2012, Shukla et al., 2011), superior longitudinal fasciculus (Cheng et al., 2010, Shukla et al., 2011, Bakhtiari et al., 2012, Barnea-Goraly et al., 2010), inferior fronto-occipital fasciculus (Jou et al., 2011b, Bakhtiari et al., 2012, Shukla et al., 2011), inferior longitudinal fasciculus (Jou et al., 2011b, Cheon et al., 2011, Bakhtiari et al., 2012, Shukla et al., 2011), frontal corona radiata (Barnea-Goraly et al., 2010), forceps major and minor (Bakhtiari et al., 2012), internal capsule (Cheng et al., 2010, Shukla et al., 2011, Barnea-Goraly et al., 2010) and corticospinal tract (Jou et al., 2011b, Bakhtiari et al., 2012, Shukla et al., 2011). Reduced FA appeared to be characterised by increased MD (Shukla et al., 2011, Gibbard et al., 2013, Kleinhans et al., 2012, Ameis et al., 2011, Cheon et al., 2011), increased RD (Shukla et al., 2011, Cheng et al., 2010, Gibbard et al., 2013, Kleinhans et al., 2012, Ameis et al., 2011) and reduced AD (Barnea-Goraly et al., 2010, Cheng et al., 2010). Collectively these findings indicate that there may be

reduced structural connectivity in commissural, association and projection fibers in individuals with ASD, and these changes may be driven by demyelination and greater axonal degeneration.

Other studies however have identified increased FA in ASD in a number of studies including the corpus callosum (Weinstein et al., 2011a, Billeci et al., 2012), superior longitudinal fasciculus (Weinstein et al., 2011a, Cheng et al., 2010), arcuate fasciculus (Billeci et al., 2012), cingulum (Weinstein et al., 2011a, Billeci et al., 2012), internal and external capsule (Billeci et al., 2012, Cheng et al., 2010), superior corona radiata, anterior thalamic radiation, inferior occipito-frontal fasciculus, middle cerebellar peduncle (Cheng et al., 2010), optic radiation and superior fronto-occipital fasciculus (Bode et al., 2011). It appears that this increase in FA is associated with reduced RD (Weinstein et al., 2011a, Cheng et al., 2010) and increased AD (Cheng et al., 2010). With the exception of two of the studies cited above, increased FA was primarily demonstrated in young children with ASD. This finding substantiates the reports of atypical neural development observed in biological studies.

Evidence suggests that gender, age and IQ impact white matter microstructural development in typically developing individuals (Clayden et al., 2012, Lebel and Beaulieu, 2011) therefore findings from white matter studies of ASD may also be affected by these factors. From the TBSS literature, it appears as though FA is increased in children aged 6 and under followed by reduced FA into adolescence and adulthood in ASD. However, some of these studies included both genders (Kleinhans et al., 2012, Gibbard et al., 2013, Bakhtiari et al., 2012, Shukla et al., 2011, Barnea-Goraly et al., 2010, Ameis et al., 2011) and were not matched for IQ (Billeci et al., 2012,

Barnea-Goraly et al., 2010) thus findings may have been influenced by this methodological variation.

1.5.3.2.2 Diffusion Tensor Imaging Tractography Studies of ASD

Using ROI DTI-tractography methods, abnormal white matter structural connectivity has been demonstrated in ASD in a number of major white matter tracts including the inferior fronto-occipital fasciculus (McGrath et al., 2013a, Kumar et al., 2010, Chang et al., 2014) arcuate fasciculus (Fletcher et al., 2010, Kumar et al., 2010, Lo et al., 2011, Roberts et al., 2014), inferior longitudinal fasciculus (Chang et al., 2014, Wolff et al., 2012), uncinate fasciculus (Kumar et al., 2010, Lo et al., 2011), cingulum (Kumar et al., 2010, Lo et al., 2011, Weinstein et al., 2011a, Ikuta et al., 2014) and the corpus callosum (Hong et al., 2011, Weinstein et al., 2011a, Kumar et al., 2010) though the results of these studies do not provide a clear picture of the white matter pathophysiology. Some of the studies have reported increased FA in ASD relative to controls (Weinstein et al., 2011a, Wolff et al., 2012) while others report reduced FA across white matter tracts (Kumar et al., 2010, Lo et al., 2011, McGrath et al., 2013a, Chang et al., 2014, Ikuta et al., 2014). Given that white matter development changes across the lifespan (Lebel et al., 2008), the varied age groups examined in both TBSS and tractography studies are likely to have influenced results. However results differ within studies utilising the same age group. For example, in adolescents, Fletcher et al. (2010) found white matter differences in the arcuate fasciculus while another study which employed constrained spherical deconvolution (CSD) tractography (an approach developed to overcome the limitations of DTI) did not (McGrath et al., 2013a) thus emphasising the importance of technical considerations when interpreting results. Notably, Lebel et al. (2008) demonstrated that the developmental trajectory of white

matter differs across tracts in neurotypical individuals thus highlighting the importance of considering the age range of the cohort studies in addition to the specific tract in studies of white matter architecture in ASD.

A number of recent studies have attempted to investigate fcMRI and DTI findings in the same ASD population (Delmonte et al., 2013, Mueller et al., 2013, Nair et al., 2013, Kana et al., 2012, McGrath et al., 2013b) however these studies have also yielded variable results. Mueller et al. (2013) identified reduced functional connectivity between the temporoparietal junction and frontal regions and reduced fractional anisotropy in the same regions. Kana, Nair and their colleagues also found reduced FA in tracts that may connect regions demonstrating weaker functional connectivity (Kana et al., 2012, Nair et al., 2013). In another study, increased functional connectivity was observed between the striatum and multiple brain regions but no difference in structural connectivity was revealed (Delmonte et al., 2013). The study by McGrath et al. was the only study to investigate the specific white matter tracts connecting neural regions showing reduced functional connectivity. This study demonstrated that reduced functional connectivity between occipito-thalamic and occipito-striatal regions was associated with reduced FA in the white matter tracts that directly connected these regions. Together, these studies support the hypothesis that there is an association between abnormal structural and functional connectivity (McGrath et al., 2013a).

1.6 Cognitive Theories of ASD

Researchers have speculated that abnormal cortical connectivity may provide the underlying framework to link proposed cognitive theories of ASD (Just et al., 2012,

Kana et al., 2011) such as impaired theory of mind (Baron-Cohen et al., 1985), reduced social motivation (Dawson et al., 2002), enhanced perceptual functioning (Mottron et al., 2006), weak central coherence (Frith and Happe, 1994) and executive dysfunction (Turner, 1999). According to the impaired theory of mind hypothesis, individuals with ASD are unable to attribute mental states such as beliefs, desires and intentions to others thus impacting on social communication and interaction. The medial prefrontal cortex, temporoparietal junction and posterior superior temporal sulcus are considered neural correlates of theory-of-mind processing and disrupted connectivity of these regions has been illustrated (Kana et al., 2009, Kana et al., 2012). However, theory of mind ability is not believed to develop until the third year of life therefore cannot explain the social deficits from birth and is also considered dependent on environmental factors and the development of other cognitive states including attention, visual processing and memory formation (Korkmaz, 2011). The social motivation theory holds that motivational factors reduce attention to social stimuli, which subsequently impairs emotional perception and social cognition. A network of neural regions including the amygdala, striatum, orbitofrontal and ventromedial prefrontal cortex are considered to modulate social motivation (Chevallier et al., 2012), all of which have demonstrated interrupted cortical connectivity (Delmonte et al., 2013, Monk et al., 2010, Murphy et al., 2012). Although these theories provide attractive neurocognitive explanations of ASD pathology, neither addresses the restrictive repetitive features of the disorder. According to the enhanced perceptual functioning hypothesis ASD is characterised by enhanced low-level processing primarily driven by greater modulation of posterior regions. This theory has been linked with the weak central coherence theory which speculates that individuals with

ASD have a processing bias for component or local information rather than global assessment (Happe and Frith, 2006). Both these theories provide an explanation of attentional features observed in ASD such as circumscribed interests and intense focus on abstract objects or object components thus, aberrant cortical connectivity within posterior regions or between frontal-posterior regions may explain these theories (Wass, 2011). Executive function, primarily facilitated by the prefrontal cortex, incorporates higher-order cognitive processes such as cognitive flexibility, attention, inhibition, impulse control, working memory and planning which continue to develop into mid-adolescence (Luna et al., 2004). Disrupted executive function in ASD is thought to underpin the restricted repetitive behavioural traits described in the disorder (Hill, 2004, Russo et al., 2007, Schmitz et al., 2006) however some studies have demonstrated intact executive functioning (Hill, 2004, Sachse et al., 2013). Luna and colleagues found that although impaired executive functioning persists into adulthood, evidence of both atypical and typical developmental progression of these processes is present in ASD, which may account for the discrepancy in findings (Luna et al., 2007).

While these theories collectively encompass the fundamental core deficits in ASD, due to the heterogeneity of the disorder, it is unlikely that a single cognitive account will explain the plethora of autistic traits reported. The abnormal cortical connectivity theory provides a plausible and compelling hypothesis for the neurobiological underpinnings of ASD that unifies a number of the rather heterogeneous theories of autism that co-exist in the current literature.

1.7 Attention

Attention dysfunction was first described in Kanner's initial report of autism and has since been one of the most consistently reported deficits in ASD. Atypical visual tracking and attention between birth and 6 months followed by atypical attention to stimuli between 6 and 12 months are two of the earliest markers for the development of ASD (Deconinck et al., 2013) and it has been suggested that the cognitive traits of ASD such as impaired theory of mind, weak central coherence and executive dysfunction may be a consequence of fundamental deficits in attentional processing (Ames and Fletcher-Watson, 2010). Furthermore, researchers have speculated that early aberrant attention modulation may contribute to the development of core social and communication deficits in ASD (Belmonte and Yurgelun-Todd, 2003, Dawson et al., 2004).

The process of attention is comprised of three neuro-functional networks; the alerting, orienting and executive control networks (Posner and Petersen, 1990). The alerting network is responsible for increasing and maintaining awareness in response to incoming information in general (tonic alertness) or in response to a specific stimulus (phasic alertness) (Fan et al., 2012, Keehn et al., 2010). The orienting network supports the selection of specific information from various sensory inputs and is comprised of three systematic operations; disengaging attention from its current focus, adjusting attention to a novel object and engaging attention at a new target (Posner et al., 1984). The executive control network of attention modulates higher-order cognitive processes such as planning, cognitive flexibility, response inhibition and conflict resolution to achieve goals or efficient cognitive functioning (Fan et al.,

2012, Keehn et al., 2010). Each of these attentional components is considered facilitated by distinct neural networks (Fan et al., 2005). The thalamus and the temporoparietal junction (TPJ), caudate, putamen and other parietal regions are thought to modulate alerting and process warning signals (Fan et al., 2007, Fan et al., 2005). Orienting is underpinned by activation of the dorsal and ventral attention networks which encompass the frontal eye fields, superior parietal lobule, intraparietal sulcus, TPJ, precuneus, inferior frontal gyrus and superior temporal sulcus (Corbetta and Shulman, 2002) while executive control is facilitated by the anterior cingulate and dorsolateral prefrontal cortices (Matsumoto and Tanaka, 2004).

In the present study, functional connectivity of the dorsal and ventral networks during attention orienting is investigated in light of the atypical cortical connectivity theory. Impaired attention orienting has been widely reported in ASD (Elsabbagh et al., 2013, Keehn et al., 2010, Vlamings et al., 2005) and is considered a crucial component of joint attention, which develops between the ages of 8 to 15 months (Meindl and Cannella-Malone, 2011). Joint attention is defined as the ability to coordinate attention between an object and a person in a social context (Mundy et al., 1986). Impairments in joint attention are noted among the earliest signs of ASDs (Leekam et al., 2000, Osterling and Dawson, 1994, Whalen et al., 2006, Bruinsma et al., 2004) and are evidenced by a distinct lack of behaviours such a pointing, coordinated eye-gaze and showing objects. Establishment of joint attention is required for socio-emotional development as it promotes both verbal and non-verbal communication (Gillespie-Lynch et al., 2012, Alessandri et al., 2005, Charman, 2003) and is also believed to be an important precursor in the development of the theory-of-mind mechanism (Korkmaz, 2011) which incorporates the cognitive skills to manage and develop relationships by

promoting the appreciation that individuals may have different opinions, beliefs, intentions, emotions and desires to our own.

Studies of attention orienting have focused on two main aspects; social attention orienting and spatial attention orienting. As described, individuals with ASD exhibit a lack of initiating social behaviours such as reduced eye-contact, pointing and sharing of attention and gaze monitoring (Adrien et al., 1993, Charman, 2003). Researchers hypothesise that these deficits are due to an inability to attribute significance to the social properties of eye-gaze (Ristic et al., 2005, Pierno et al., 2006) while others suggest that these deficits are due to a general spatial attention orienting impairment (Chawarska et al., 2003, Leekam et al., 2000, Ames and Jarrold, 2007, Allen and Courchesne, 2001). Modification of the attention orienting paradigm developed by Posner and colleagues has advanced our understanding of both social and spatial orienting deficits in ASD (Posner et al., 1984). This paradigm involves reacting to a target that appears on either the left or right side of a screen. Prior to the appearance of the target, an informative cue indicates which side of the screen the target will be presented. In the majority of these trials, the cue will direct attention towards the same position where the target will appear (valid trials) while in the remaining trials, the cue will direct attention away from the correct target position (invalid trials). In the latter trials, participants are required to disengage and re-adjust their attention in the correct direction thus increasing reaction time and providing a performance measure, namely the 'validity effect'.

For the most part, studies utilising the Posner paradigm adopted arrow-cues and eye-gaze cues to probe social orienting using this paradigm. Results revealed that individuals with ASD orient their attention reflexively in response to both cue types

(Chawarska et al., 2003, Senju et al., 2004, Vlamings et al., 2005) However, while they react comparably slower than controls for both cue types, typically developing individuals show a cueing preference in response to eye-gaze cues (Senju et al., 2004; Vlamings et al., 2005). Furthermore, studies have demonstrated that ASD cohorts only show a cueing effect of eye-gaze during valid trials but not during invalid trials (Ristic et al., 2005; Chawarska et al., 2003). These researchers suggest that this is due to a lack of appreciation for the social significance of eye-gaze thus the ASD cohort are not distracted by them. Leekam, Ames and colleagues illustrated that ASD populations had equal difficulty orienting towards and identifying a target in response to a non-human cue and a human cue suggesting that attention orienting deficits can be attributed to more than a lack of appreciation for the social benefits of eye-gaze (Leekam et al., 2000; Ames & Jarrold, 2007).

Studies investigating if a general attention orienting deficit can explain irregular social interactions in ASD have shown atypical disengagement and shifting of attention in the absence of social cues (Hill, 2004, Landry and Bryson, 2004, Townsend et al., 1999a, Wainwright-Sharp and Bryson, 1993). Researchers have indicated that impaired attention orienting in ASD may be due to the disruption of two attentional mechanisms; voluntary goal-driven top-down 'endogenous' attention (Haist et al., 2005, Wainwright-Sharp and Bryson, 1993) and/or reflexive involuntary bottom-up 'exogenous' attention (Renner et al., 2006, Townsend et al., 1996). Conversely, other studies have demonstrated that both endogenous (Senju et al., 2004) and exogenous (Iarocci and Burack, 2004) attention are intact in ASD. Methodological issues such as cue–target timing, specific type of cue used, appropriate distinction of attentional mechanisms and perceptual load are thought to influence results thus should be

considered when designing attention orienting paradigms [see (Ames and Fletcher-Watson, 2010) for review].

Collectively, genetic, neuropathological and neuroimaging literature provides support for the abnormal cortical connectivity theory of ASD. While many studies have investigated this theory in a number of cognitive functions and white matter tracts, many avenues of research have yet to be explored. Development of advanced neuroimaging methods can allow for more in-depth analyses of grey and white matter cortical structure. These improved methodological approaches must be implemented to gain a greater understanding of the pathology of ASD.

1.8 Thesis Rationale

Attention orienting is a cognitive process that facilitates the movement of attentional focus from one location to another in response to a stimulus. Attention orienting is likely to be essential for the development of social functioning due to its role in joint attention. While impaired attention orienting has been described, the precise neurobiological mechanisms underpinning this impairment have not yet been established. Given the growing body of evidence supporting the atypical cortical connectivity theory of ASD, implementing functional connectivity analyses to investigate the functional neural networks that underpin attention orienting may further elucidate the nature of these impairments. Additionally, given the large number of studies that have described atypical functional connectivity between fronto-parietal regions in addition to the role of these regions in attention and executive functioning, it is surprising that the microstructural organisation of the major

white matter tract structurally connecting these regions, the superior longitudinal fasciculus, has not yet been studied using tractography methods in ASD.

With the development of new methodological approaches, it is important to build on previous findings of structural abnormalities in ASD and identify consistent patterns of structural irregularity. In addition, research must strive to dissect structural anomalies with the primary aim of identifying neural biomarkers to aid early ASD diagnosis, which is crucial to augment adaptive functioning. As ASD is a neurodevelopmental disorder and neural development is not considered complete until adulthood, it is important to understand how structural abnormalities develop across the lifespan in the disorder as they may offer potential novel therapeutic targets. Moreover, additional research focused on understanding the relationship between brain structure and behaviour may guide choice of therapeutic intervention and provide neural biomarkers to evaluate these interventions in the future.

1.9 Aims

1. To investigate functional connectivity of the dorsal and ventral attention networks during attention orienting between individuals with ASD and age and IQ matched typically developing individuals
2. To isolate the three fronto-parietal branches of the superior longitudinal fasciculus using a novel analytic approach - constrained spherical deconvolution based tractography - in a population of ASD participants and controls in order to examine white matter architectural differences between the groups
3. To explore differences in whole-brain white matter organisation using high angular resolution diffusion imaging data from ASD and control participants

4. To evaluate the derivatives of cortical volume, cortical thickness and surface area in addition to their related measures, gyrification and sulcal depth, in a sample of ASD and neurotypical individuals
5. To determine if the developmental trajectory of cortical components diverges in individuals with ASD relative to typically developing individuals
6. To assess the relationship between behavioural characteristics, social and communication deficits (SCD) and restricted repetitive behaviours (RRB) and structural abnormalities in ASD

2 Methods

This chapter outlines the protocols used throughout the following studies. A description of ethical approval, recruitment, diagnostic screening instruments, sample characteristics, the basic principals of magnetic resonance imaging (MRI), MRI data acquisition and the statistic analyses used are explained in the following paragraphs.

2.1 Ethical Approval

Ethical approval was obtained from the Irish Health Services Executive Linn Dara Child and Adolescent Research Ethics Committee, St. James's Hospital / The Adelaide and Meath National Children's Hospital and the School of Psychology Ethics Committee Trinity College Dublin.

2.2 Recruitment

Right-handed males aged between 10 and 25 with autism spectrum disorders were recruited from an existing autism genetics sample in Trinity College Dublin as well as through schools, universities, advocacy groups and child and adolescent mental health services. Both ASD and control participants were recruited through schools, universities, social media sites, volunteer websites, local businesses and educational programmes. Exclusion criteria for the ASD cohort included below average intelligence (full-scale IQ < 70), disorders associated with autism such as Fragile X syndrome, loss of consciousness, serious head injuries, MR contraindications, comorbid neurological or psychological disorders or current use of psychoactive medication (See Appendix A for Initial Contact Form). Claustrophobia was also a consideration for exclusion. For the

control cohort, additional exclusion criteria included a history of developmental delay, a score > 12 on the social communication questionnaire (SCQ), a score > 50 on the social responsiveness scale (SRS) or first-degree relatives with a diagnosis of an autism spectrum disorder. Written consent was obtained from all participants aged over 18 years. Both parental written consent as well as written assent from participants was obtained from all participants under 18 years. Participants were given instructions and the opportunity to practice the neuropsychological task prior to scanning. These instructions were repeated directly before the task was performed in the scanner. All participants were given tokens of gratitude after the scanning session was completed. Following the MRI scan, subjects were excluded if there was excessive motion or incomplete scan sessions.

The data reflected in the current work was derived from three sources referred to as the 'McGrath' sample, 'Delmonte' sample and the 'Fitzgerald' sample. All three samples were assembled for three PhD projects under the supervision of Prof. Louise Gallagher. The 'McGrath' sample was recruited by Dr. Jane McGrath, co-supervisor of the current research. The 'Delmonte' sample was recruited by Dr. Sonja Delmonte and the 'Fitzgerald' sample was recruited by the author. There was some overlap in participants recruited across the studies. Therefore, if an individual was scanned twice, the highest quality scan i.e. the scan with the least head motion, was selected for inclusion. The purpose of utilising all three samples in the current thesis was to increase power to facilitate a number of analyses not previously conducted (Chapters 4, 5, 6). Details of participants included in each study are outlined in Table 2.1.

Table 2.1. Description of Data Sources

	3T			Participants included			
Collected by	**Scanning Site**	**Instruments used**	**No. of Participants**	**Study 1**	**Study 2**	**Study 3**	**Study 4**
Dr. Jane McGrath	TCIN	ADI, ADOS					
Control		WASI	25	21	24	24	23
ASD		WISC-III	25	21	24	24	23
Sonja Delmonte PhD	CAMI	ADI, ADOS					
Control		WASI	27				18
ASD		SRS, SCQ	28				18
Jacqueline Fitzgerald	TCIN	ADI, ADOS					
Control		WASI	24		21	21	22
ASD		SRS, SCQ	26		21	21	22

TCIN = Trinity Institute of Neuroscience, Trinity College Dublin, CAMI = Centre for Advanced Medical Imaging (CAMI), St. James's Hospital, ADI = Autism Diagnostic Interview, ADOS = Autism Diagnostic Observation Schedule, WISC = Wechsler Intelligence Scale for Children, WASI = Wechsler Abbreviated Scale of Intelligence, SRS = Social Responsiveness Scale, SCQ = Social Communication Questionnaire, Study 1 = Functional Connectivity Analysis during Attention Orienting (Chapter 3), Study 2 = CSD Tractography of the Superior Longitudinal Fasciculus (Chapter 4), Study 3 = Whole Brain Investigation of White Matter Structure (Chapter 5), Study 4 = Whole Brain Investigation of Grey Matter Cortical Structure (Chapter 6)

2.3 Diagnostic Screening

2.3.1 Autism Diagnostic Interview-Revised

The Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994) and the Autism Diagnostic Observation Schedule-Generic (ADOS-G) (Lord et al., 2000) were used together to confirm a research standard clinical diagnosis of autism spectrum disorder (ASD) for all ASD participants prior to recruitment. The Autism Diagnostic Interview–Revised (ADI-R) is a structured interview conducted with parents or caregivers and focuses on both previous and current behaviour. The ADI-R consists of 93 questions exploring developmental history, language functioning, communication, social development, interests and clinically relevant behaviours. The diagnostic algorithm consists of four subdomains; qualitative abnormalities in reciprocal social interaction, qualitative abnormalities in communication, restricted, repetitive and stereotyped patterns of behaviour and abnormality of development evident before 36 months. Participants must score above the cut-off in each behavioural sub-domain to receive a clinical diagnosis of ASD, and this was the criterion used in the current study.

2.3.2 Autism Diagnostic Observation Schedule-Generic

The Autism Diagnostic Observation Schedule-Generic (ADOS-G) is a semi-structured diagnostic observational assessment developed for the research setting, which enables the examiner to observe the social interaction, social communication and repetitive behaviours of the participants. The ADOS-G contains four modules, one of which is selected for the assessment based on prior knowledge of the participants age and expressive language. Module four is most suitable for verbal older children,

adolescents and adults with fluent speech. The diagnostic algorithm has a three-step cut-off. Individuals must score at least 2 on the communication sub-domain, a minimum of 4 on the reciprocal social interaction sub-domain and at least a score of 7 for the combined scores of the sub-domains to receive a diagnosis of ASD. A score of 3 on communication, 6 on reciprocal social interaction and a combined score of 10 on both domains will yield a diagnosis of autism. Scores of stereotyped behaviours and restricted interest are also recorded but are not required for clinical diagnosis.

2.3.3 Intelligence Quotient

The Wechsler Abbreviated Scale of Intelligence (WASI; (Wechsler, 2004) was used to evaluate intelligence quotient (IQ) for both ASD and controls participants. The WASI consists of four subtests; vocabulary, similarities, block design and matrix reasoning which collectively provide a measure of full scale IQ. Verbal IQ can be ascertained from the vocabulary and similarities subtests. The vocabulary subtest measures word knowledge and verbal concept formation while the similarities subtest measures verbal reasoning and concept formation. Performance IQ can be established from the block design and matrix reasoning subtests. The block design subtest assesses nonverbal concept formation, visual perception and visual-motor coordination while the matrix reasoning subtest assesses abstract reasoning in addition to visual information processing.

2.3.4 Social Responsiveness Scale

The Social Responsiveness Scale (SRS) is a clinically validated 65-item questionnaire for measuring ASD characteristics that have been present within the past six months (Constantino et al., 2003). The SRS can also be used to evaluate

autistic traits in the general population. The SRS scoring worksheet consists of five sub-domains; awareness, cognition, communication, motivation and mannerisms. T-scores above 70 on the SRS are indicative of ASD. The parent report SRS form was completed by one of the participant's parents, for both cases and controls for use as a quantitative measure and also to exclude the presence of ASD in controls. As the SRS was not collected in all cohorts, it was not used as a tool to investigate behavioural correlations with neuroimaging findings.

2.3.5 Social Communication Questionnaire

The Social Communication Questionnaire (SCQ) is a 40-item questionnaire derived from the ADI-R (Rutter et al., 2003). Scores of above 15 on the SCQ indicate that a diagnosis of ASD is likely. The SCQ was completed for both cases and controls although primarily the purpose was to exclude ASD symptoms in controls.

2.4 Basic Principles of Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a non-invasive method that can provide images of the brain by utilising the magnetic properties of atomic nulcei such as the hydrogen nucleus which is present in all body tissues. Different image types can be acquired based on the relaxation properties of the nuclei as well as the application of different pulse sequences and imaging parameters (Huettel et al., 2009).

For neuroimaging, multiple 2D slices of the brain are acquired in the axial plane and are reconstructed into 3D imaging space (Huettel et al., 2009). The resulting 3D image can be viewed in any plane (i.e. axial, coronal or sagittal). The basic units of these images are voxels, or three dimensional volume elements, which vary in size

from 1 to 2mm for structural MRI (high spatial resolution) and 3 to 5 mm for functional MRI (low spatial resolution). In this research, 3 image acquisitions were performed. A brief description of each type is outlined below.

2.4.1 Structural MRI

By modifying scanning sequence parameters such as repetition time (TR) and echo time (TE), anatomical images can determine the contrast of brain tissue. Anatomical structure can be estimated with the acquisition of T_1-weighted scans. These scans can measure the relaxation time of nuclei within various tissues such as fat and water, which differs depending on neural tissue type. T_1-weighted images can distinguish between grey and white matter tissue therefore T_1-weighted scans are used to generate high-resolution structural images of the brain to investigate differences in anatomical structure.

2.4.2 Functional MRI

Functional MRI (fMRI) permits the study of the neural functioning that underpins cognitive processes. T_2*-weighted images use a sequence which shows increased contrast between tissue and venous blood. T_2*-weighted images form the basis of fMRI as they can detect changes in regional blood flow. Neurons rely on surrounding astrocytes for the provision of metabolites and nutrients. When a cognitive, sensory or motor process causes an increase in neural activation in certain brain regions, there is an increased demand for energy. This leads to increases in blood flow to the relevant neural regions where oxygenated blood displaces deoxygenated blood. fMRI methods are designed to measure changes in the inhomogeneity of the magnetic field that results from changes in blood oxygenation. The blood-oxygen-level-dependent (BOLD)

contrast provides an index of neural activity by using the difference in signal on T_2*-weighted images. Areas with greater BOLD signal represent brain regions of increased neuronal activation implying their involvement in the specific cognitive, sensory or motor process under study (Huettel et al., 2009).

The BOLD response occurs in three phases. A slight decrease in image intensity below baseline during the initial period of oxygen absorption is followed by a steep increase above baseline reaching peak levels. This is due to the blood flow over-compensating for the increased energy demand. This is followed by a sub-baseline decrease after the oversupply of oxygenated blood has been reduced. It is thought that peak blood flow occurs at approximately 6 seconds and the overall BOLD response returns to baseline after 20 seconds (Huettel et al., 2009). The canonical haemodynamic response function (HRF), a standard measure of the rapid delivery of blood to active neuronal tissue, is generally used to model the BOLD signal and can provide an indirect measure of neuronal activation in a voxel.

2.4.3 Diffusion MRI

Diffusion MRI can provide information about white matter microstructural changes in vivo (Basser et al., 2000, Jones et al., 1999, Jones and Leemans, 2011). Diffusion MRI manipulates the properties of water molecules within brain tissue to generate structural images. The mobility of water molecules is driven by constant thermal agitation known as Brownian motion (Jones, 2008). If there are no restrictions to water movement such as cell membranes, water will diffuse equally in all directions, known as isotropic diffusion. However, if there are barriers to diffusion, such as myelinated axons of nerves, the water molecules diffuse primarily in one direction,

known as anisotropic diffusion. The magnetic gradients applied during diffusion MRI detect the directionality of water molecule diffusion (Huettel et al., 2009) thus, white matter tracts are discernible using Diffusion MRI methods due to their highly anisotropic diffusion properties.

The diffusion tensor model is the most commonly used methodological approach for analysis of diffusion MRI data. This model consists of a 3x3 matrix for each voxel (Basser et al., 1994a, Basser et al., 1994b) composed of three eigenvalues (λ_1, λ_2, λ_3) and corresponding eigenvectors ($\hat{e}_1$, $\hat{e}_2$, $\hat{e}_3$). The eigenvalues provide the magnitude of diffusion while the eigenvectors demonstrate the direction of diffusion. The first eigenvalue (λ_1) represents parallel or axial diffusivity (AD). The average of the second and third eigenvalues (λ_2 and λ_3) illustrates perpendicular or radial diffusivity (RD). Mean diffusivity (MD) is the average diffusion of all three eigenvalues. Fractional anisotropy (FA) is calculated based on the three eigenvalues using the following formula;

$$\mathrm{FA} = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

An FA value of 0 indicates that diffusion is isotropic and an FA value of 1 indicates that diffusion is anisotropic. Changes in FA may be a consequence of changes in axial or radial diffusivity or a combination of these metrics. Fractional anisotropy is thought to reflect white matter microstructure in terms of myelination, axonal organisation and fiber density. Radial diffusivity is believed to represent myelination of axons while axial diffusivity is thought to reflect axonal degeneration (Song et al., 2002).

Crossing or 'kissing' fiber pathways thought to be present in 90% of voxels in the brain (Jeurissen et al., 2013) are known to influence diffusion derived metrics

(Basser et al., 2000, Jones, 2003, Alexander et al., 2002) and can hinder 'true' interpretation of DTI data (Vos et al., 2011). Constrained spherical deconvolution (CSD) is a methodological approach developed to overcome issues in tractography analyses due to voxels containing multiple fiber orientations (Tournier et al., 2007). CSD tractography can manage partial volume effects and provide reliable diffusivity estimates by facilitating fiber tracking through complex neural regions (Tournier et al., 2008) thus generating more robust tract reconstructions.

2.5 MRI Data Acquisition

All scanning was conducted on a Philips Intera Achieva 3.0 Tesla MR system (Best, The Netherlands) equipped with a mirror which was projected onto a panel placed behind the participants' head outside the magnet. The mirror was mounted on the head coil in the participants' line of vision. The mirror reflected a visual display (projection of a computer screen) which presented the stimuli and images for task performance.

2.5.1 Structural T_1-Weighted Acquisition Parameters

A parallel Sensitivity Encoding (SENSE) approach (Pruessmann et al., 1999) with a reduction factor of 2 was utilized for T1-weighted image acquisitions. 180 high-resolution T1-weighted anatomic MPRAGE axial images (FOV = 230 x 230 x 162 mm3; thickness 0.9 mm3; voxel size 0.9mm3; total acquisition time = 343s) were then acquired to allow subsequent activation localisation and spatial normalization.

Acquisition for the 'Delmonte' cohort differed slightly. For this population, high-resolution T_1-weighted MPRAGE image was acquired (FOV = 256 x 256 x 160 mm3;

thickness 1mm3; voxel size 1mm3; total acquisition time = 450s). As this data was acquired using a slightly different protocol in a different scanner, it was included as a covariate in analyses that included this cohort.

2.5.2 Functional T_2*-Weighted Acquisition Parameters

Functional data were collected using a T_2*-weighted echoplanar imaging sequence that acquired 39 non-contiguous slices covering the entire brain (TE = 30 ms; TR=2000 ms, FOV = 224 x 224 x 149 mm3; voxel size = 3.5 x 3.5 x 3.5 mm3; slice thickness = 3.5 mm; slice gap = 0.35mm; matrix size 64 x 64 mm2 in Fourier space). The functional scans had a total duration of 148s per run and there were 6 runs.

2.5.3 Diffusion MRI Acquisition Parameters

Diffusion weighted data were acquired using a single-shot echo-planar imaging (EPI) sequence SENSE approach. Diffusion-weighted images were encoded along 61 independent directions (TR = 20244 ms; TE = 79ms ; B-value = 1500; slice thickness = 2 mm; slice gap = 0 mm; FOV = 248 x 248 130 mm^3; number of slices = 65; voxel size = 1.94 x 1.94 x 2 mm^3; total acquisition time = 24mins 21.7 secs) and one non-diffusion weighted image was acquired.

2.6 Statistical Analyses

2.6.1 Functional Connectivity Analysis

Functional MRI data were preprocessed and analysed using AFNI (http://afni.nim.nih.gov/afni/; (Cox, 1996) and FSL (FMRIB Software Library – http://www.fmrib.ox.ac.uk/fsl/). IBM SPSS Statistics, Version 19 (Armonk, NY: IBM

Corp) was used to investigate between-group task performance (see Chapter 3, Section 3.3.3 for more details).

2.6.2 Diffusion Magnetic Resonance Imaging Analyses

Preprocessing of diffusion data was completed using ExploreDTI software (http://www.ExploreDTI.com) (Leemans et al., 2009). CSD based tractography was performed prior to isolation of the Superior Longitudinal Fasciculus. IBM SPSS Statistics, Version 19 (Armonk, NY: IBM Corp) was used to perform between-group analyses of diffusion metrics (see Chapter 4, Section 4.3 for more details).

2.6.3 Tract Based Spatial Statistical Analyses

As with the tractography analysis, preprocessing of diffusion data was completed using ExploreDTI software (http://www.ExploreDTI.com) (Leemans et al., 2009) (see Chapter 4, Section 4.3.2 for more details). Whole brain voxel-wise analysis of white matter integrity was performed using Tract-based Spatial Statistics (Smith et al., 2006) within FSL FMRIB Software (Smith et al., 2004) version 4.1.9 (see Chapter 5, Section 5.3.3 for more details).

2.6.4 Grey Matter Cortical Structure Analyses

Preprocessing of T_1-weighted structural images was performed using Freesurfer image analysis software, version 5.3.0 (http://surfer.nmr.mgh.harvard.edu). Vertex-by-vertex group differences were evaluated using QDEC, an application within the Freesurfer software package (see Chapter 6, Section 6.3 for more details).

2.6.5 Behavioural Measures used for Correlation Analyses

Most neuroimaging studies to date have used ADI-R and/or ADOS-G total scores as well as SRS total scores for investigating the link between neural abnormalities and symptom severity. However, these measures are clinically heterogeneous and provide ranked non-normally distributed data. Furthermore, it remains unclear how these scores relate to the underlying neurobiology of ASD thus translation of research findings into clinical practice has been difficult. The ADI-R is composed of three domains; social interaction, communication and restricted repetitive behaviours, while the ADOS-G is primarily concerned with social interaction and communication and does not take into account restricted repetitive behaviours in the total score. With the development of the DSM-V, greater emphasis has been placed on a two factor model consisting of social and communication deficits (SCD) and restricted repetitive behaviours (RRB). A number of factor analytic studies have provided support for the two-factor model (Boomsma et al., 2008, Frazier et al., 2008, Georgiades et al., 2013, Mandy et al., 2012). Importantly, factor analysis based on the ADI-R has biological validity as it has been shown to be heritable in genetic studies (Liu et al., 2011a). The current study used the two-factor model developed by Georgiades et al. (2013) to aid our understanding of neurobiological heterogeneity in ASD. Georgiades and colleagues selected 26 algorithm items that apply to all individuals regardless of age or verbal ability. For the model, 20 items were loaded on the SCD domain and the remaining six loaded onto the RRB domain (Appendix B). Factor scores for each domain were calculated for every individual in the current study for use in correlational analyses to investigate the association between neural abnormalities and symptomology in ASD. Correlation analyses were performed using IBM SPSS Statistics, v19 (Armonk, NY: IBM).

3 Functional Connectivity of Attention Orienting

3.1 Introduction

3.1.1 Attention Orienting

Attention orienting, a cognitive process that facilitates the movement of attentional focus from one location to another in response to a stimulus, involves three steps; disengaging, shifting and re-engaging attention (Posner et al., 1984). Deficits in attention orienting (Elsabbagh et al., 2013, Renner et al., 2006, Vlamings et al., 2005, Keehn et al., 2010), as well as specific deficits in disengaging (Landry and Bryson, 2004, Wainwright-Sharp and Bryson, 1993) and shifting (Hill, 2004, Townsend et al., 1996, Townsend et al., 1999a) attention have been repeatedly reported in autism spectrum disorders. Impairment in attention orienting occurs in response to both social (Dawson et al., 2004, Leekam et al., 2000, Ristic et al., 2005, Dawson et al., 1998) and non-social stimuli (Dawson et al., 1998, Dawson et al., 2004, Renner et al., 2006). It has been suggested that social deficits characteristic of ASDs may be underpinned by atypical attention orienting (Haist et al., 2005, Nation and Penny, 2008). Recent research investigating attention abnormalities in ASD has suggested that attentional deficits may precede social deficits and represent the earliest signs of ASD (Orekhova and Stroganova, 2014, Elsabbagh et al., 2013). It is therefore vital to understand the neural underpinnings of attention orienting dysfunction in ASD.

3.1.2 Attention Orienting Paradigm

The Posner cueing task is a behavioural paradigm used to establish the neural basis of attention orienting (Doricchi et al., 2010, He et al., 2007, Kincade et al., 2005,

Corbetta and Shulman, 2002, Corbetta et al., 2002, Corbetta et al., 2000). This paradigm involves instructing participants to respond to a target, which appears on either the left or right of a screen. The target is preceded by a cue, which offers information about the location of the target. This information can be 'valid', i.e. where it provides information on the correct spatial location of the target or 'invalid', i.e. where it provides incorrect information regarding the location of the target. Additionally, the task includes non-informative neutral trials and cue-only catch-trials. The most commonly used measure to evaluate behavioural performance is the 'Validity Effect', which measures the response time benefit of the provision of a valid cue compared with the provision of an invalid cue.

3.1.3 Attention Orienting in ASD

Clinically, abnormal attention orienting has been widely reported in ASD (Dawson et al., 2004, Leekam and Ramsden, 2006), and researchers have used the Posner paradigm to study this deficit. A number of these studies have demonstrated that attention orienting is impaired in ASD (Renner et al., 2006, Dawson et al., 2004, Leekam and Ramsden, 2006, Landry and Bryson, 2004, Townsend et al., 1996, Harris et al., 1999, Haist et al., 2005), whereas others have reported intact attention orienting in ASD (Fan et al., 2012, Pruett et al., 2011, Kuhn et al., 2010, Iarocci and Burack, 2004, Kylliainen and Hietanen, 2004, Senju et al., 2004, Chawarska et al., 2003). Researchers have suggested that this variability in results may be due to methodological issues (see (Ames and Fletcher-Watson, 2010) for review) and it has also been hypothesised that individuals with ASDs may employ atypical neural mechanisms to achieve typical behaviour (Fan et al., 2012, Greene et al., 2011). An investigation of the neural

correlates of attention orienting in ASD is essential to understand the pathophysiology of aberrant attention orienting in individuals with ASD.

3.1.4 Neural Correlates of Attention Orienting – Dorsal and Ventral Attention Networks

Functional MRI and resting state functional connectivity analyses have demonstrated that two interacting neural networks subserve attention orienting: the dorsal attention network (DAN) and the right hemisphere ventral attention network (VAN) (Shulman et al., 2010, Shulman et al., 2009, Kincade et al., 2005, He et al., 2007, Corbetta and Shulman, 2011, Corbetta and Shulman, 2002, Corbetta et al., 2000, Fox et al., 2006, Fox and Raichle, 2007). The DAN comprises the intraparietal sulcus (IPS) and part of the frontal cortex at the human homologue of the frontal eye field (FEF). It modulates voluntary, goal-driven top-down 'endogenous' attention (Kincade et al., 2005, Corbetta and Shulman, 2002, Corbetta et al., 2000, Thiel et al., 2004, Hopfinger et al., 2000) and is engaged, for example, when attention is cued by the appearance of an arrow. The DAN can be investigated using the cue-only trials of the Posner task at cue onset – it is at this time point that goal-driven attention is engaged. The VAN consists of a right dominant cortical network involving the temporoparietal junction (TPJ) and the ventral frontal cortex (VFC) including the middle and inferior frontal gyri, frontal operculum and the anterior insula (Shulman et al., 2010, Shulman et al., 2009, Vossel et al., 2006, Thiel et al., 2004, Macaluso et al., 2002, Arrington et al., 2000). The VAN modulates bottom-up 'exogenous' attention, which involuntarily directs attention in an automatic reflexive manner to an unexpected stimulus such as the appearance of a target. The VAN is believed to act as a 'circuit breaker' for the dorsal attention

network, as it disengages attention from one location and directs it towards the new object of interest (Shulman et al., 2009, Kincade et al., 2005, Corbetta et al., 2008, Vossel et al., 2006). Presentation of the target during valid and invalid trials in the Posner task modulates the VAN, with greater activation of the temporoparietal junction (TPJ), precuneus, anterior insula, middle frontal gyrus (MFG) and superior temporal sulcus (STS) during invalid trials compared with valid trials (He et al., 2007). Only a few neuroimaging studies have investigated attention orienting in ASD (Fan et al., 2012, Greene et al., 2011, Pruett et al., 2011, Keehn et al., 2010, Haist et al., 2005). While these studies have shown abnormal function in regions of the DAN and VAN, no previous study has investigated functional connectivity of the DAN and VAN in an ASD population.

3.1.5 Abnormal Cortical Connectivity Theory of ASD

Previous neuroimaging research has demonstrated numerous abnormalities in brain function and structure in autism, from which a theory of abnormal cortical connectivity in autism has emerged (see (Vissers et al., 2012) for review). This postulates that the behavioural deficits in autism are underpinned by abnormal interregional brain connectivity. Functional connectivity is a measure of the co-ordination of neural processing between brain regions (Friston, 2011). Disrupted functional connectivity has been demonstrated during performance of cognitive processes such as visuospatial processing (McGrath et al., 2012, Liu et al., 2011b, Damarla et al., 2010), cognitive control (Solomon et al., 2009, Solomon et al., 2013), response inhibition (Agam et al., 2010, Lee et al., 2009), face processing (Monk et al., 2010, Kleinhans et al., 2008), social reward processing (Delmonte et al., 2012),

sentence and language comprehension and processing (Mizuno et al., 2011, Kana et al., 2006) and working memory (Koshino et al., 2008, Just et al., 2007, Koshino et al., 2005). Behavioural deficits in ASD correlate with atypical functional connectivity (Kleinhans et al., 2008, Mizuno et al., 2011, Agam et al., 2010, Solomon et al., 2009, Delmonte et al., 2013). Patterns of connection dysfunction imply under-connectivity between long-range fronto-posterior regions, whilst patterns between short-range connections have been shown to be more variable. Some studies indicate short-range over-connectivity (Monk et al., 2010, Noonan et al., 2009, Mizuno et al., 2006, Turner et al., 2006), however several other studies have revealed short-range under-connectivity (Agam et al., 2010, Monk et al., 2010, Kleinhans et al., 2008, Turner et al., 2006). Studies of resting state networks also reveal aberrant functional connectivity (von dem Hagen et al., 2013, Weng et al., 2010, Di Martino et al., 2011, Cherkassky et al., 2006), most notably in a recent large pooled analysis by Di Martino et al. (2013).

Interestingly, many of the studies that have shown abnormal functional connectivity in ASD have identified disrupted connectivity within brain regions that are part of the DAN and VAN including the frontal eye fields (Agam et al., 2010), intraparietal sulcus (Koshino et al., 2008), temporoparietal junction (Kana et al., 2009) precuneus (Just et al., 2007, Solomon et al., 2009, Mizuno et al., 2006, Mizuno et al., 2011), middle frontal gyrus (Kana et al., 2007, Just et al., 2007, Koshino et al., 2008, Delmonte et al., 2013), anterior insula (Kana et al., 2007, Just et al., 2007, Mizuno et al., 2011) and the superior temporal sulcus (Kana et al., 2009). Although atypical functional connectivity has been demonstrated in ASD during many different cognitive functions, no previous studies have investigated functional connectivity during attention orienting.

3.1.6 Aims

Based on the paucity of neuroimaging studies examining attention orienting in ASD (Fan et al., 2012, Greene et al., 2011, Pruett et al., 2011, Keehn et al., 2010, Haist et al., 2005) and no prior investigation of functional connectivity in the two neural networks (DAN and VAN) that subserve attention orienting, we chose to investigate this in more detail in ASD. Functional connectivity of the DAN and VAN was investigated in ASD during the Posner task. Specifically, connectivity of brain regions involved in the DAN was investigated during cue-only trials at cue onset, as this is the time at which goal-driven attention is engaged. Functional connectivity of the VAN was probed during target onset on valid and invalid trials, as this is the time at which involuntary reflexive attention is modulated. Based on previously reported atypical cortical connectivity in ASD, we hypothesised that there would be abnormal functional connectivity in the ASD group relative to the control group during attention orienting in both the dorsal and ventral attention networks.

3.2 Methods

3.2.1 Participants

Participants for this study were taken from the 'McGrath' cohort. Details of the participants for this study (study 1) are described in Chapter 2, Section 2.2 and in Table 3.1.

Table 3.1. Participant Demographics

	Control	ASD	P-value
Number	21	21	
Gender	Male	Male	
Mean age ± SD; range	17.5 ± 2.7; 13.6-24.2	17.4 ± 2.8; 12.7-24.3	0.948
Mean Full Scale IQ ± SD; range	112.1 ± 16.7; 83-147	107.9 ± 14.1; 84-145	0.380
Handedness	Right	Right	
Medication	None	None	
Ethnicity	Irish	Irish	

3.2.2 Posner Spatial Attention Orienting Paradigm

The Posner attention paradigm used was based on a previous study investigating functional connectivity during attention orienting in individuals with spatial neglect (He et al., 2007). The orienting cue varied in four ways: valid, invalid, neutral and cue only. The task began with presentation of a green central fixation cross inside a white diamond shape for 900ms. The spatial orienting cue was an arrow, depicted as the

brightening of the left or right side of the diamond, and was presented for 2000ms. After presentation of the orienting cue, there was an interstimulus interval (ISI), which was jittered between 1500-3000ms. This was followed by presentation of a target (white asterisk) on either the right or left of the screen for 100ms. Participants were asked to respond as quickly as possible when the target was observed by pressing a button. Each trial ended with a red fixation cross that was presented for a time jittered between 6000ms and 7000ms. 60 valid trials and 20 invalid, cue-only and neutral trials were presented. See Figure 3.1 for a schema of the task. The total of 120 trials were presented in random order equally proportioned across 6 runs, each of which lasted 148 seconds.

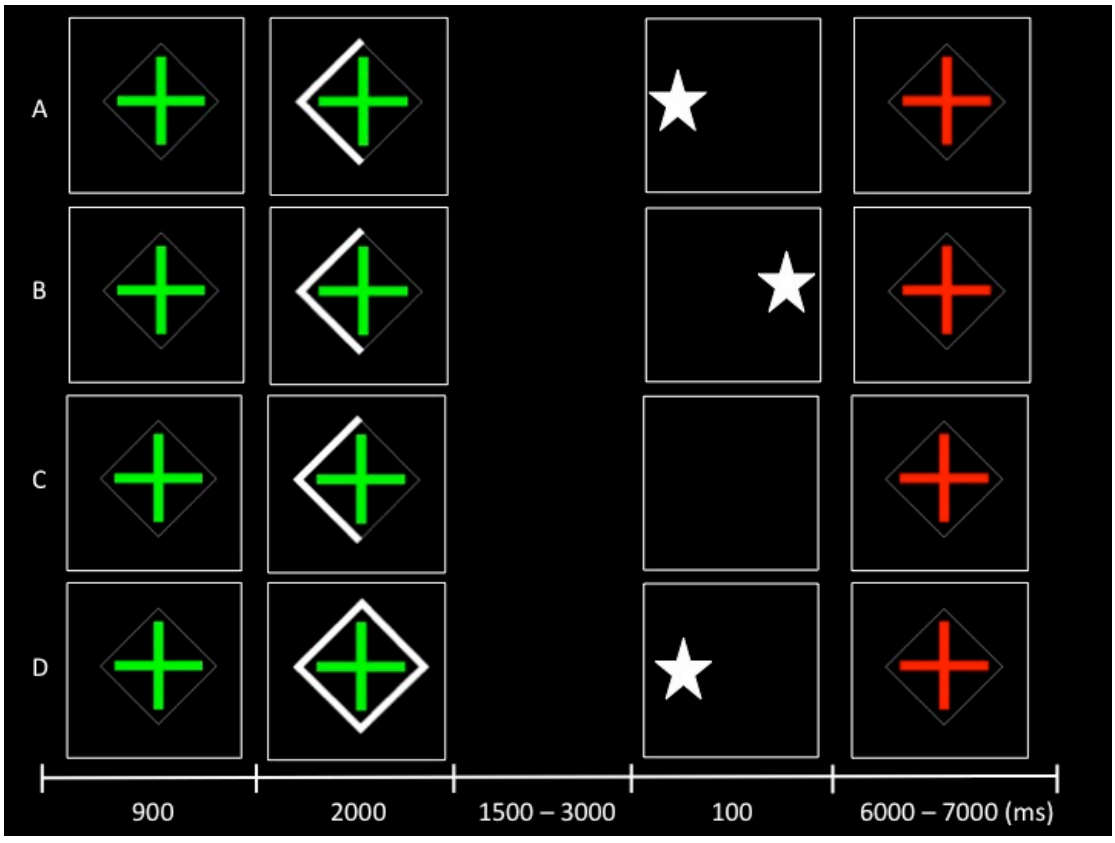

Figure 3.1. Illustration of Trial Types from the Attention Orienting Paradigm

A) Valid trial, B) Invalid trial, C) Cue-only trial, D) Neutral trial.

3.2.3 Data Analysis

3.2.3.1 Behavioural Data Analysis

Response time between target presentation and button press was isolated and averaged across each trial condition for every participant. A two-way mixed-factorial ANOVA (Group [ASD/Control] x Trial [Valid/Invalid]) was performed to determine if a validity effect was observed and to investigate if this differed between groups. Statistical threshold was set to $p < 0.05$ for all analyses and post-hoc Bonferroni corrections were performed.

3.2.3.2 Functional MRI Analysis

MRI data were analysed using AFNI (http://afni.nim.nih.gov/afni/; (Cox, 1996) and FSL (FMRIB Software Library – http://www.fmrib.ox.ac.uk/fsl/). Each run was configured into the same spatial orientation. Each run was time-shifted using Fourier interpolation and each slice was aligned to the same temporal origin. All runs were concatenated and motion corrected by realignment to the first volume of the first run. TRs with translational or rotational motion exceeding 0.9mm were removed. Data were smoothed with a 6mm full-width-at-half-maximum Gaussian kernel to reduce the effect of noise and grand-mean scaling was performed.

A general linear model was set up and a regression analysis was conducted in AFNI. Four regressors (valid, invalid, neutral, cue) of interest were included to probe the neural correlates of attention orienting. Cue-onset was modelled for cue-only trials. Target-onset was modelled for valid, invalid and neutral trials. These regressors modelled the mean BOLD signal change from baseline across all trials. Several regressors of no interest were also included to model the sources of variance including

6 motion parameters. Contrasts of all trial types against an implicit baseline were obtained.

The regressor coefficients maps were normalised into standard stereotaxic space by warping them to the MNI brain template (Montreal Neurological Institute/International Consortium for Brain Mapping 152 standard atlas) in FSL using linear registration tool, FLIRT. The 12-parameter affine transformation matrix was calculated using high-resolution structural images from each subject.

3.2.3.3 Psychophysiological Interaction Analysis

Functional connectivity analyses typically measure the synchronicity of brain regions by examining associations in BOLD intensity time series across the brain. A psychophysiological interaction (PPI) analysis (Friston et al., 1997) can be implemented to investigate task-dependent functional connectivity. In PPI analyses, functional connectivity is estimated by measuring how a selected brain region of interest interacts with the rest of the brain during specific psychological task conditions. Therefore, PPI analyses can probe how connectivity of brain regions is modulated based on task demands.

For this study, regions of interest (ROIs) were selected based on a meta-analysis of four studies of spatial attention using a Posner paradigm (He et al., 2007). In this meta-analysis, four bilateral ROIs [frontal eye fields (FEF), ventral and posterior intraparietal sulci (vIPS and pIPS) and middle temporal complex (MT+)] were found to be modulated during cue-onset indicating their role in the dorsal attention network and were therefore selected to investigate functional connectivity in the dorsal attention network during cue-onset. The meta-analysis also reported that five right

hemisphere regions [temporoparietal junction (TPJ), precuneus, anterior insula, middle frontal gyrus (MFG) and superior temporal sulcus (STS)] showed greater activation during the presentation of invalid cues relative to valid cues, implying a role in the ventral attention network. Thus these regions were selected to explore functional connectivity in the ventral attention network during invalid and valid trials.

For each ROI, an 8mm cube was converted from standard stereotaxic space into native space for each individual. The de-trended time course of the ROI was isolated and a physiological regressor was created. Stimulus onset times were not synchronized with the TR grids of 2 seconds, therefore the time-series of the ROI was upsampled by 20 and the TR grid was extended into 0.1 second segments in order to include stimuli that did not occupy complete TRs (http://afni.nimh.nih.gov/sscc/gangc/CD-CorrAna.html). The time-series was then de-convolved. A task regressor was generated that contained '1's for all sub-TRs during which the condition of interest (valid, invalid or cue-onset) occurred and '0's for all other TR's (McGrath et al., 2012, O'Reilly et al., 2012). An interaction regressor was then calculated for each condition of interest by multiplying the physiological variable, the up-sampled de-convolved time-series, by the task variable. The interaction regressor was then convolved with the haemodynamic response function (HRF) and subsequently down-sampled to TR grids. A regression analysis (as outlined in the functional MRI analysis) was conducted in AFNI for each ROI. A separate analysis was performed for each condition. These analyses included the addition of the physiological regressor, the task regressor and the interaction regressor of interest (cue-only, valid, invalid) to the general linear model discussed previously in the fMRI analysis section. A Fisher z transformation was then

performed on the interaction regressor to reduce skewness and to normalise the sampling distribution.

Co-registration of the Fisher Z transformation map to the MNI template was performed as outlined in the fMRI analysis. To investigate between-group differences in functional connectivity in the dorsal attention network, t-tests of Z-scores from PPI analyses of the cue-only condition in four bilateral ROIs were performed. To investigate between-group differences in functional connectivity in the ventral attention network, t-tests of Z-scores from target onset in a condition of interest (valid or invalid) in five right hemisphere dominant ROIs were completed. The significance level was set at a voxelwise statistical threshold of $p < 0.005$ and each brain cluster was required to be a minimum size of 708 µl. This minimum cluster size was calculated by Monte Carlo simulation to obtain a family wise error corrected value of $p < 0.05$ for statistical significance. Results of the functional connectivity analysis were subsequently masked by task activation for the condition of interest across groups to ensure that any brain regions showing abnormal functional connectivity with one of the investigated ROIs were activated by the task. Furthermore, a Bonferroni correction was carried out where the alpha level was adjusted for the number of PPI analyses performed for each ROI for each condition.

The dependent variables of the PPI analysis were positive and negative connectivity. Positive connectivity indicates that the ROI and a resulting region of activation are positively correlated; as activation increases in one ROI during the task, activation increases in the correlated region. Negative connectivity indicates that engaging in the task produces a correlated opposite effect on the correlated region, as

activation in the ROI is reduced during the task, activation in the correlated region increases. This is consistent with (but not proof of) one region suppressing the other.

3.3 Results

3.3.1 Behavioural Results

In terms of mean Response Time (MRT), there was a significant main effect of Validity, F (1, 40) = 20.026, $p < 0.001$, $\eta_p^2 = 0.334$, indicating that all participants detected and responded to the target asterisk more quickly during valid trials (358.34 ± 68.32ms) compared with invalid (381.54 ± 63.41ms) trials, $p < .001$ (Figure 3.2). There was no significant difference in MRT between the ASD and control groups, (F (1, 40) = 0.708, p = 0.405), nor was there a significant interaction between Validity and Group, (F (1, 40) = 0.603, p = 0.442).

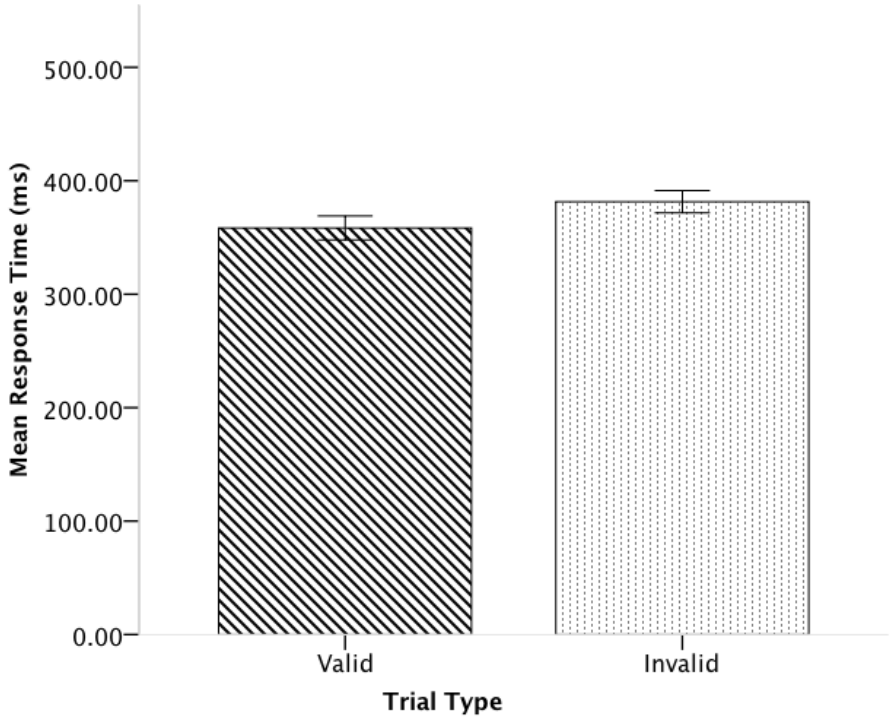

Figure 3.2. Graph of Behavioural Performance during Attention Orienting

Mean response time (ms) during invalid and trials across both ASD and control groups, corrected at $p < 0.05$.

3.3.2 Functional MRI Results

3.3.2.1 Dorsal Attention Network

Activation of the DAN was investigated during cue-onset. Regions activated corresponded with literature published in neurotypical populations (Kincade et al., 2005, Corbetta et al., 2000, Hopfinger et al., 2000) (Figure 3.3).

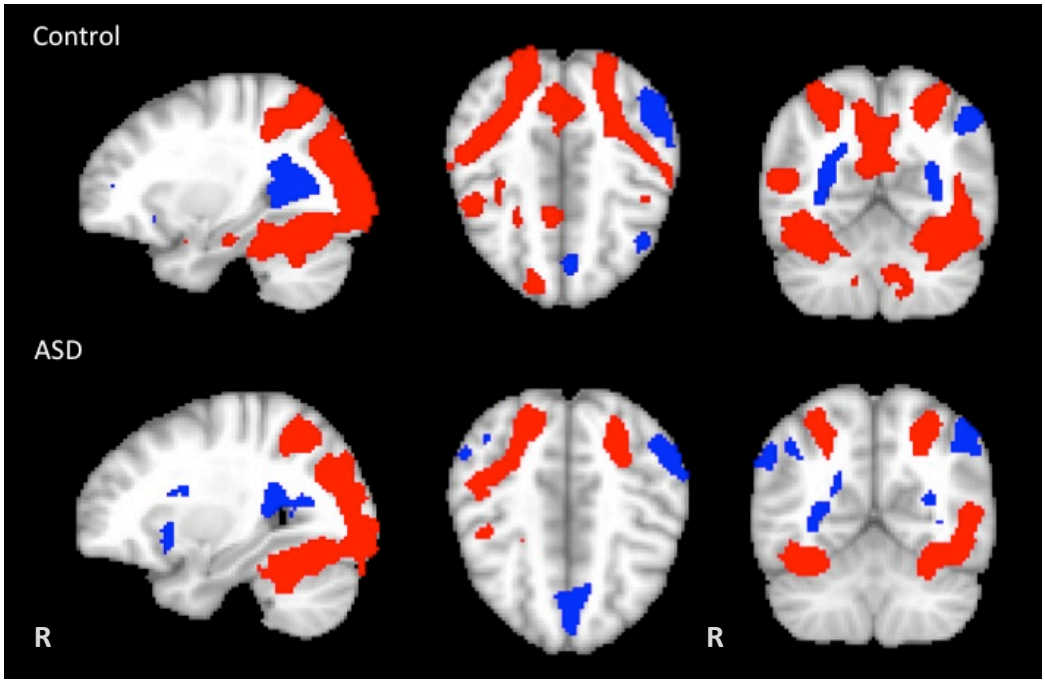

Figure 3.3. BOLD Activation during Cue-Onset for ASD and Control Groups

Activation maps illustrate activation (red) in several brain regions including bilateral occipital regions, precuneus, inferior and superior parietal lobules, middle and posterior cingulate cortex and supramarginal gyrus. Deactivation (blue) was observed in the right angular gyrus and right inferior and middle superior frontal gyri and the bilateral orbital cortex. Data corrected for multiple comparisons, $p < 0.05$.

3.3.2.2 Ventral Attention Network

Activation of the VAN was explored during invalid and valid trials (Figure 3.4). Regions activated corresponded with literature published in neurotypical populations (Hopfinger et al., 2000, Corbetta et al., 2000, Kincade et al., 2005, Vossel et al., 2006).

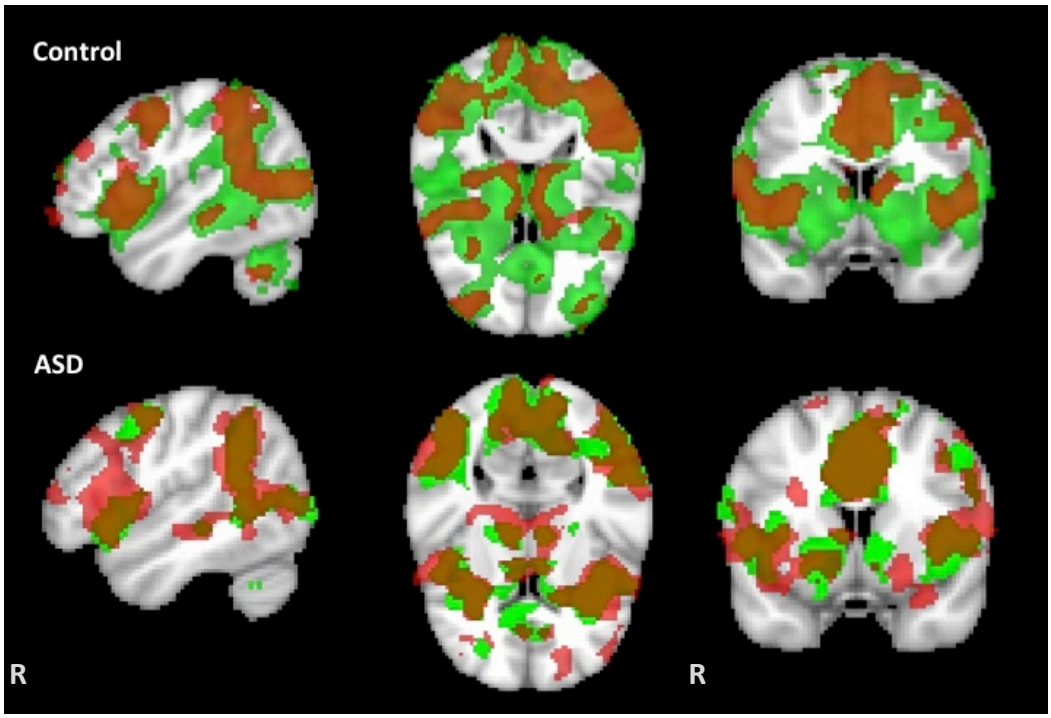

Figure 3.4. BOLD Activation during Valid and Invalid Trials for ASD and Control Groups

Activation during orienting to a validly cued target (green) as well as reorienting to a target following an invalid cue (red) was observed in bilateral frontal, temporal, parietal, occipital and cerebellar regions in both groups. Brown colour illustrates the overlap in regions activation during both trial conditions. Data corrected for multiple comparisons, $p < 0.05$.

3.3.3 Psychophysiological Interaction Results

3.3.3.1 Dorsal Attention Network

Eight PPI analyses were performed to examine functional connectivity of the DAN during cue-onset using four bilateral ROIs that are considered part of the DAN; these include the frontal eye field, ventral intraparietal sulcus, posterior intraparietal sulcus and the middle temporal complex. Four of the eight PPI analyses demonstrated significant differences between the ASD and control groups, corrected for multiple comparisons, $p < 0.05$ (Table 3.2 and Figure 3.5). Compared with controls, the ASD group showed much weaker functional connectivity between ROIs and connected regions. These results are described in detail below.

3.3.3.1.1 Right Frontal Eye Fields

Functional connectivity between the right frontal eye field ROI and the left hippocampus and fusiform gyrus demonstrated that the ASD group exhibited positive connectivity while the control group demonstrated negative functional connectivity (Table 3.2 and Figure 3.5).

3.3.3.1.2 Left Ventral Intraparietal Sulcus

The control group demonstrated stronger negative connectivity between the left ventral intraparietal sulcus and the left middle temporal gyrus relative to the ASD group (Table 3.2 and Figure 3.5).

3.3.3.1.3 Right Ventral Intraparietal Sulcus

The ASD group demonstrated negative connectivity whilst positive connectivity was observed in the control group between the right ventral intraparietal sulcus and the left fusiform gyrus (Table 3.2 and Figure 3.5).

3.3.3.1.4 Left Posterior Intraparietal Sulcus

The control group demonstrated stronger negative connectivity between left posterior intraparietal sulcus and the left middle temporal gyrus, parahippocampal gyrus and fusiform gyrus relative to the ASD group (Table 3.2 and Figure 3.5).

ROI Regions significantly functionally connected to the ROI

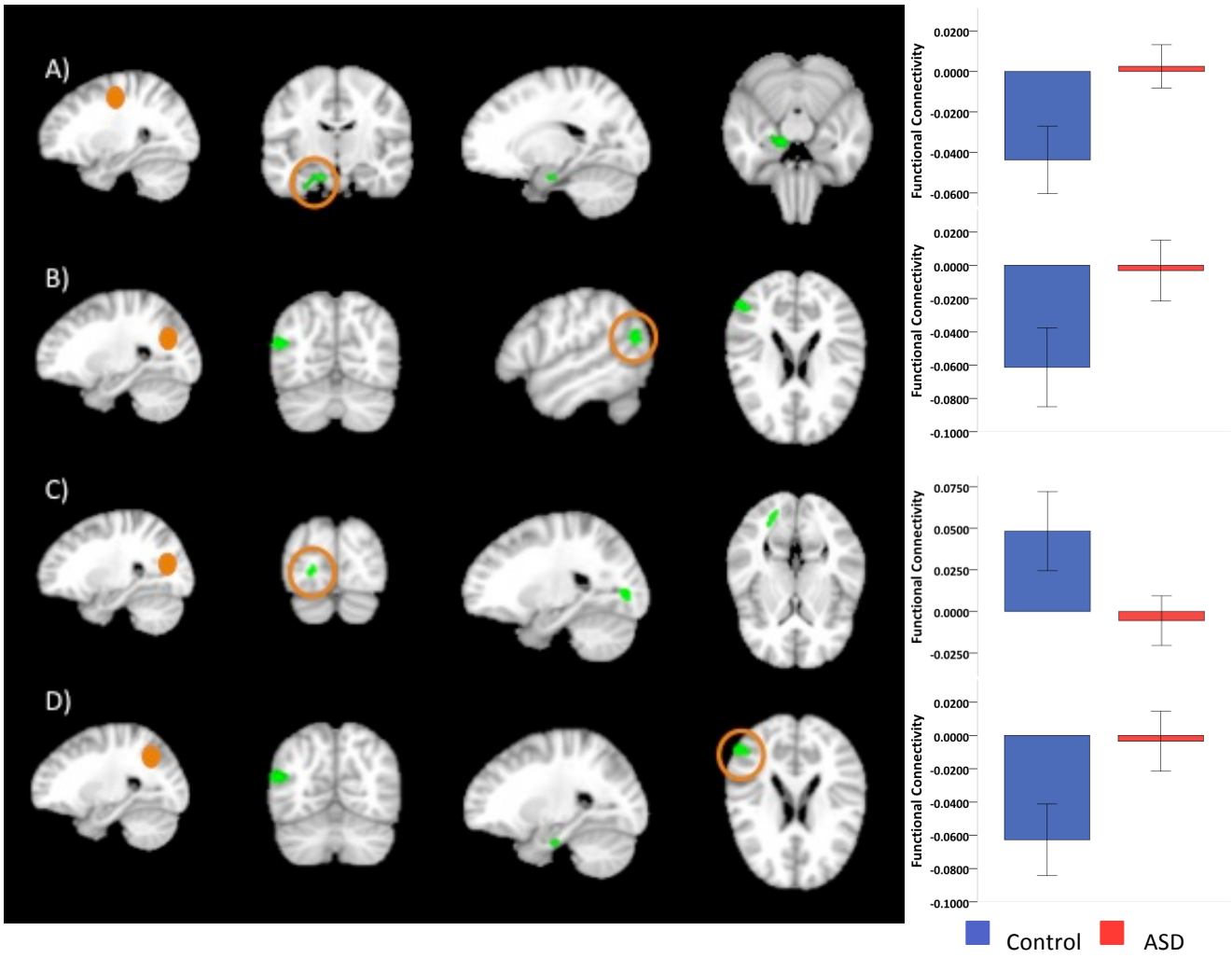

Figure 3.5. Group Differences in Functional Connectivity during Cue-Onset

Four of the eight PPI analyses showed between group differences. The circled green clusters illustrate the peak region (highest number of voxels) connected to the ROI (shown as a solid orange sphere). Graphs represent the strength and direction (positive or negative) of functional connectivity of the peak region functionally connected to each ROI for both the ASD and control groups; A) Right Frontal Eye Field B) Left Ventral Intraparietal Sulcus C) Right Ventral Intraparietal Sulcus D) Left Posterior Intraparietal Sulcus. R = Right, L = Left.

Table 3.2. Regions of Functional Connectivity during Cue-Onset

	Seed ROI		Connected region	X	Y	Z	T-value	Connectivity
R	Frontal Eye Field	L	Hippocampus	-14	-12	-24	4.96	C – ASD +
		L	Fusiform Gyrus	-28	-14	-34	3.41*	C – ASD +
L	Ventral Intraparietal Sulcus	L	Middle Temporal Gyrus	-54	-68	20	4.06	C - > ASD -
R	Ventral Intraparietal Sulcus	L	Fusiform Gyrus	-22	-84	2	4.07	C + ASD -
L	Posterior Intraparietal Sulcus	L	Middle Temporal Gyrus	-54	-66	18	4.41	C - > ASD -
		L	Parahippocampal Gyrus	-24	-16	-28	3.78	C - > ASD -
		L	Fusiform Gyrus	-28	-14	-34	3.66	C - > ASD -

Direction of between-group difference is denoted with arrows (- = negative connectivity, + = positive connectivity), > = greater than (note that when both groups show negative connectivity the > means a larger negative value), X, Y, Z = Peak Montreal Neurological Institute (MNI) Co-ordinates, R = Right, L = Left, C = Control group, ASD = Autism Spectrum Disorder group]. *Did not survive Bonferroni correction for the adjusted p-value of 0.00625.

3.3.3.2 Ventral Attention Network

Five PPI analyses were performed to investigate functional connectivity in the VAN during invalid and valid trials. ROIs that had been identified as part of the VAN, the right temporoparietal junction, right precuneus, right middle frontal gyrus, right anterior insula and right superior temporal sulcus, were used to investigate functional connectivity of the VAN during valid and invalid trials separately. During both invalid

and valid trials a significant difference in functional connectivity between groups was observed across all five PPI analyses, corrected for multiple comparisons, $p < 0.05$ (Table 3.3). The main pattern emerging during **invalid** trials was that the ASD group demonstrated significant positive functional connectivity to multiple brain regions whilst the control group demonstrated negative connectivity. This pattern was observed in four out of the five analyses with the exception of the superior temporal sulcus analysis (Table 3.3 and Figure 3.6). During **valid** trials, two patterns of functional connectivity were apparent. In the first pattern the control group demonstrated negative connectivity but the ASD group demonstrated positive connectivity (Table 3.4). In the second pattern, the ASD group demonstrated reduced negative connectivity in contrast to controls (Table 3.4 and Figure 3.7).

3.3.3.2.1 Right Temporoparietal Junction

During **invalid** trials, positive connectivity was demonstrated in the ASD group between the right temporoparietal junction (TPJ) ROI and the bilateral anterior cingulate, bilateral middle frontal gyrus and right inferior and superior frontal gyrus whereas the control group demonstrated negative functional connectivity between these regions (Table 3.3 and Figure 3.6). During **valid** trials, stronger negative connectivity was demonstrated in controls in comparison to the ASD group in projections between the TPJ and parietal (right superior and inferior parietal lobule) regions (Table 3.4 and Figure 3.7). Additionally during valid trials, stronger negative connectivity was demonstrated in the control group relative to the ASD group between the right insula, right cuneus, the left supplementary motor area (SMA) and the TPJ. Projections between the TPJ and the right inferior frontal gyrus demonstrated stronger negative connectivity in controls relative to ASD whereas connectivity between the left

inferior frontal gyrus and the TPJ demonstrated that the ASD group demonstrated positive connectivity but the control group demonstrated negative connectivity.

3.3.3.2.2 Right Precuneus

During **invalid** trials, several regions demonstrated a pattern of opposite connectivity (bilateral anterior cingulate cortex, bilateral middle frontal gyri, right superior medial, mid orbital and inferior frontal gyri and the right cerebellum) where the ASD group demonstrated positive connectivity and the control group demonstrated negative connectivity (Table 3.3 and Figure 3.6). During **valid** trials, stronger negative connectivity was demonstrated in controls relative to the ASD group between the right precuneus and regions in the frontal lobe (bilateral inferior frontal gyrus), left insula and posterior region of the right precuneus (Table 3.4 and Figure 3.7). Additionally, projections between this right precuneus ROI and parietal regions (right superior and inferior parietal lobule), right supramarginal gyrus, thalamus, anterior cingulate, left fusiform and middle frontal gyri demonstrated an opposite pattern of connectivity characterised by positive connectivity in the ASD group and negative connectivity in controls.

3.3.3.2.3 Right Middle Frontal Gyrus

During **invalid** trials, the ASD group demonstrated positive connectivity between right middle frontal gyrus ROI and the bilateral anterior cingulate and bilateral middle frontal gyri in contrast to the control group, which demonstrated negative connectivity between the right middle frontal gyrus and these regions (Table 3.3 and Figure 3.6). One exception was the projection between two regions of the right middle frontal gyrus, which were positively connected in both groups, but the ASD group

demonstrated stronger positive connectivity. During **valid** trials, stronger negative connectivity was demonstrated in the control group relative to the ASD group between the right middle frontal ROI and the right superior parietal lobule (Table 3.4 and Figure 3.7). The ASD group demonstrated positive functional connectivity between the right middle frontal gyrus and the left inferior frontal gyrus and insula whereas the control group demonstrated negative connectivity.

3.3.3.2.4 Right Anterior Insula

During **invalid** trials, the ASD group demonstrated positive functional connectivity between the anterior insula and the right middle frontal gyrus, bilateral anterior cingulate and the right cerebellum whereby the controls demonstrated negative connectivity (Table 3.3 and Figure 3.6). During **valid** trials, connectivity between the anterior insula ROI and the left inferior frontal and fusiform gyri was positive in the ASD group and negative in the control group (Table 3.4 and Figure 3.7). An exception to this pattern was demonstrated in connections between the anterior insula ROI and the right superior parietal lobule where the control group demonstrated stronger negative connectivity relative to controls.

3.3.3.2.5 Right Superior Temporal Sulcus

During **invalid** trials, the control group demonstrated positive functional connectivity between the right superior temporal sulcus ROI and the right cerebellum and fusiform gyrus in contrast to the ASD group, which demonstrated negative connectivity (Table 3.3 and Figure 3.6). During **valid** trials, the right superior temporal sulcus demonstrated reduced negative functional connections to the right inferior and superior parietal lobules as well as the right inferior frontal gyrus and insula in the ASD

group relative to controls (Table 3.4 and Figure 3.7). During valid trials, the ASD group demonstrated positive connectivity between the right STS in regions in the left hemisphere (left inferior frontal gyrus and insula lobe) whereas the control group demonstrated negative connectivity. During valid trials, positive connectivity was also demonstrated in projections to/from the superior temporal sulcus ROI and the right cuneus in the ASD group but the control group demonstrated negative connectivity.

ROI Regions significantly functionally connected to the ROI

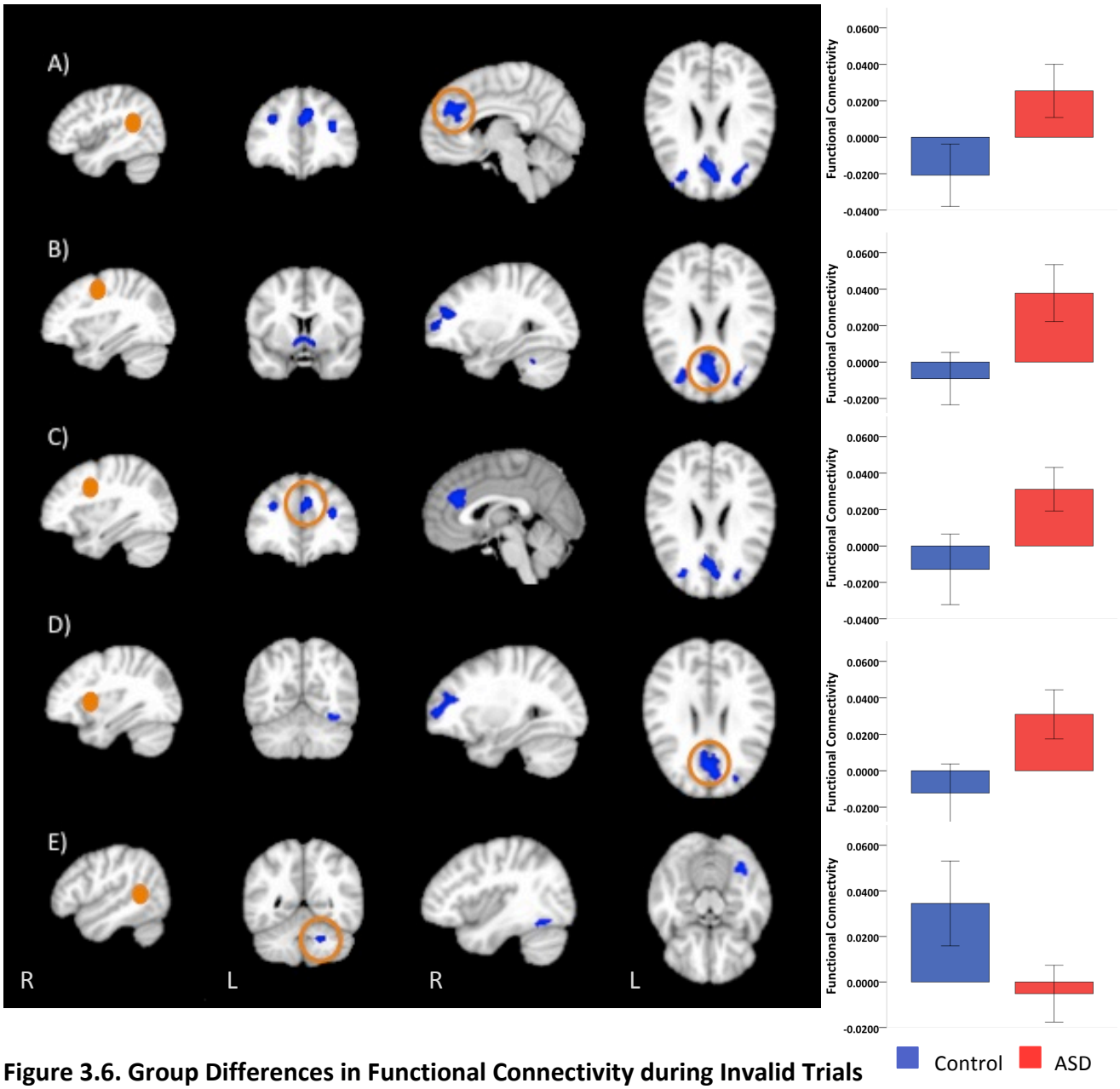

Figure 3.6. Group Differences in Functional Connectivity during Invalid Trials

The circled regions (blue) illustrate the peak region (highest number of voxels) connected to the ROI (shown as an orange sphere). Graphs represent the strength and direction (positive or negative) of functional connectivity of the peak region functionally connected to each ROI for both the ASD and control groups; A) Right Temporoparietal junction B) Right Precuneus C) Right Middle Frontal Gyrus D) Right Anterior Insula E) Right Superior Temporal Sulcus. R = Right, L = Left.

Table 3.3. Regions of Functional Connectivity during Invalid Trials

	Seed ROI		Connected region	X	Y	Z	T-value	Connectivity
R	Temporoparietal Junction	R	Anterior Cingulate	8	44	28	4.39	C – ASD +
		L	Anterior Cingulate	0	36	26	4.18	C – ASD +
		R	Middle Frontal Gyrus	32	38	20	4.34	C – ASD +
		R	Superior Frontal Gyrus	26	46	26	4.22	C – ASD +
		R	Inferior Frontal Gyrus	40	30	32	4.07	C – ASD +
		L	Middle Frontal Gyrus	-30	44	26	3.94	C – ASD +
R	Precuneus	L	Anterior Cingulate	-4	32	24	4.65	C – ASD +
		R	Anterior Cingulate	8	44	28	4.18	C – ASD +
		R	Superior Medial Gyrus	12	42	6	3.19*	C – ASD +
		R	Superior Orbital Gyrus	30	58	2	3.18*	C – ASD +
		R	Middle Frontal Gyrus	32	40	18	4.17	C – ASD +
		R	Inferior Frontal Gyrus	40	30	32	3.8	C – ASD +
		L	Middle Frontal Gyrus	-28	42	26	4.61	C – ASD +
		R	Cerebellum	18	-54	-34	4.16	C – ASD +
R	Middle Frontal Gyrus	R	Anterior Cingulate	8	42	28	4.09	C – ASD +
		L	Anterior Cingulate	0	34	20	3.86	C – ASD +
		R	Middle Frontal Gyrus	32	38	30	4.13	C + < ASD +
		L	Middle Frontal Gyrus	-30	44	26	4.05	C – ASD +
R	Anterior Insula	R	Anterior Cingulate	8	42	28	4.4	C – ASD +
		L	Anterior Cingulate	2	34	28	4.36	C – ASD +
		R	Middle Frontal Gyrus	30	56	6	4.35	C – ASD +
		R	Cerebellum	18	-52	-34	4	C – ASD +
R	Superior Temporal Sulcus	R	Cerebellum	36	-62	-16	3.66	C + ASD –
		R	Fusiform Gyrus	32	-60	-14	3.58	C + ASD –

Direction of between-group difference is denoted with arrows (- = negative connectivity, + = positive connectivity), < = less than, X, Y, Z = Peak Montreal Neurological Institute (MNI) Co-ordinates, R = Right, L = Left, C = Control group, ASD = Autism Spectrum Disorder group. *Did not survive Bonferroni correction for the adjusted p-value of 0.01.

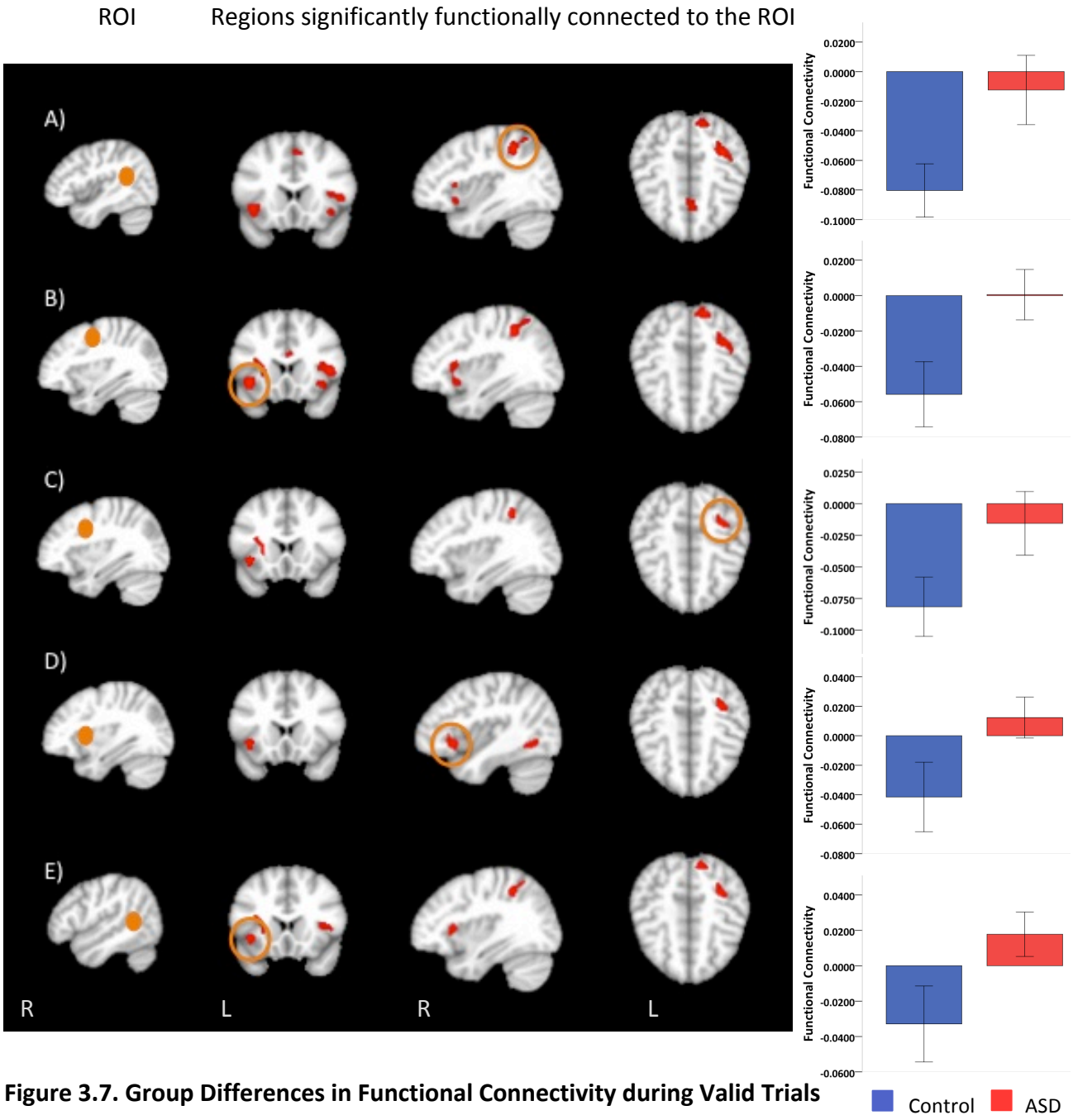

Figure 3.7. Group Differences in Functional Connectivity during Valid Trials

The circled regions (red) illustrate the peak region (highest number of voxels) connected to the ROI (shown as an orange sphere). Graphs represent the strength and direction (positive or negative) of functional connectivity of the peak region functionally connected to each ROI for both the ASD and control groups; A) Right temporoparietal junction B) Right Precuneus C) Right Middle Frontal Gyrus D) Right Anterior Insula E) Right Superior Temporal Sulcus. R = Right, L = Left.

Table 3.4. Regions of Functional Connectivity during Valid Trials

Seed ROI		Connected region	X	Y	Z	T-value	Connectivity
Temporoparietal Junction	R	Superior Parietal Lobule	36	-44	46	4.91	C – > ASD –
	R	Inferior Parietal Lobule	36	-54	56	3.57	C – > ASD –
	L	Inferior Frontal Gyrus	-40	22	-6	4.77	C – ASD +
	R	Inferior Frontal Gyrus	46	22	4	4.43	C – > ASD –
	R	Insula	36	20	8	3.41	C – > ASD –
	R	Cuneus	16	-72	44	4.11	C – > ASD –
Precuneus	L	Inferior Frontal Gyrus	-38	2	-6	5	C – > ASD –
	L	Insula	-32	18	16	3.48	C – > ASD –
	R	Inferior Frontal Gyrus	46	22	6	5.15	C – > ASD –
	R	Superior Parietal Lobule	36	-44	46	4.66	C – ASD +
	R	Inferior Parietal Lobule	38	-54	56	3.64	C – ASD +
	R	Supramarginal	44	-36	48	3.44	C – ASD +
	R	Thalamus	16	-12	4	4.06	C – ASD +
	R	Precuneus	16	-74	46	4.6	C – > ASD –
	L	Fusiform Gyrus	-42	-60	-6	3.95	C – ASD +
	L	Middle Frontal Gyrus	-38	34	40	3.84	C – ASD +
	R	Anterior Cingulate	8	24	28	3.28	C – ASD +
	L	Precuneus	-6	-42	62	3.52	C – ASD +
Middle Frontal Gyrus	R	Superior Parietal Lobule	34	-44	46	4.12	C – > ASD –
	L	Insula	-32	18	16	3.41	C – ASD +
	L	Inferior Frontal Gyrus	-38	22	-6	4.07	C – ASD +
Anterior Insula	L	Inferior Frontal Gyrus	-38	22	-6	4.23	C – ASD +
	R	Superior Parietal Lobule	34	-44	46	4.45	C – > ASD –
	L	Fusiform Gyrus	-42	-60	-6	3.59	C – ASD +

Superior Temporal Sulcus	L	Inferior Frontal Gyrus	-38	22	-6	4.36	C – ASD +
	L	Insula	-32	18	16	3.52	C – ASD +
	R	Superior Parietal Lobule	34	-44	46	4.17	C – > ASD –
	R	Inferior Parietal Lobule	32	-52	50	3.65	C – > ASD –
	R	Cuneus	14	-72	44	4.41	C – ASD +
	R	Inferior Frontal Gyrus	46	22	4	3.79	C – > ASD –
	R	Insula	36	20	8	3.57	C – > ASD –

Direction of between-group difference is denoted with arrows (- = negative connectivity, + = positive connectivity), > = greater than (note that when both groups show negative connectivity the > means a larger negative value), X, Y, Z = Peak Montreal Neurological Institute (MNI) Co-ordinates, R = Right, L = Left, C = Control group, ASD = Autism Spectrum Disorder group. All survived Bonferroni correction for the adjusted p-value of 0.01.

3.4 Discussion

3.4.1 Overview of Findings

The primary aim of this study was to investigate functional connectivity in the dorsal and ventral attention networks during attention orienting in ASD. This study found that despite similar response times and functional MRI activation, there were three key between-group differences in functional connectivity. (1) In the DAN, relative to controls, the ASD group showed significantly weaker functional connectivity between ROIs and connected regions. (2) In the VAN, during **invalid** trials, the ASD group showed stronger positive functional connectivity whilst the control group showed stronger negative functional connectivity. (3) In the VAN, during **valid** trials, the ASD group showed a mixed pattern of results. Whilst the control group showed strong negative functional connectivity, the ASD group showed strong positive functional connectivity between some areas but weak negative functional connectivity between other regions of the VAN. The weaker functional connectivity shown by the ASD group in the DAN suggests weaker coherence between brain areas involved in goal-driven, endogenous attention control. The strong positive functional connectivity exhibited by the ASD group in the VAN during the invalid trials suggests that individuals with ASD may generate compensatory mechanisms to achieve neurotypical behaviour. These results support the theory of abnormal cortical connectivity in autism and demonstrate that individuals with ASD may engage different neural mechanisms to the neurotypical population to perform attention orienting.

3.4.2 Typical Performance of Attention Orienting in ASD

The behavioural and functional MRI analyses demonstrated that the adolescents with ASD performed the Posner cueing task in a similar manner to their neurotypical counterparts. As with previous literature, behavioural results showed that a validity effect was observed across both groups during task performance (Pruett et al., 2011, Renner et al., 2006, Vossel et al., 2006, Thiel et al., 2004, Townsend et al., 1999b, Townsend et al., 1996, Townsend et al., 1999a, Harris et al., 1999). This indicates that the task effectively probed attention orienting and the ASD group was able to perform the task to the same standard as the control group. This finding is consistent with previous behavioural findings investigating attention orienting in ASD using this paradigm (Fan et al., 2012, Pruett et al., 2011, Kuhn et al., 2010, Iarocci and Burack, 2004, Kylliainen and Hietanen, 2004, Senju et al., 2004, Chawarska et al., 2003).

3.4.3 Functional Connectivity of the Dorsal Attention Network

Activation of the DAN was investigated during cue-onset. Regions activated corresponded with literature published in neurotypical populations (Kincade et al., 2005, Corbetta et al., 2000, Hopfinger et al., 2000). Functional connectivity analysis revealed significant disruption in connectivity in the DAN in the ASD group relative to controls.

As mentioned above, the ASD group showed reduced functional connectivity relative to controls in the DAN. In both groups, modulation of the DAN during cue onset activated occipital and parietal regions as expected based on previous literature (Damarla et al., 2010, Lee et al., 2007, Manjaly et al., 2007). However the functional connectivity analysis revealed interesting between-group differences in that the ASD

group showed significantly weaker functional connectivity relative to controls between ROIs (left and right ventral and posterior IPS and right frontal eye fields) and functionally connected regions in the left hemisphere (left hippocampus, middle temporal, parahippocampal and fusiform gyri). This disrupted functional connectivity in the ASD group may provide a neurobiological explanation for the clinical deficits in endogenous goal-driven attention orienting that have been reported in individuals with ASD (Ristic and Kingstone, 2005).

3.4.4 Functional Connectivity of the Ventral Attention Network

The VAN is activated during target presentation in the Posner task, and brain regions activated in both groups during target presentation in this study were consistent with previous literature probing VAN modulation (Corbetta and Shulman, 2002). Functional connectivity analysis revealed significant disruption in connectivity in the VAN in the ASD group relative to controls.

3.4.4.1 Functional Connectivity during Invalid Trials

During invalid trials there was abnormal connectivity between regions of the VAN and connections to/from the cingulate, cerebellar and frontal regions. These between-group differences in functional connectivity between the VAN and anterior cingulate were particularly interesting. Four of the five ROIs (right TPJ, precuneus, MFG and anterior insula) used to interrogate the VAN showed strong positive functional connectivity with the anterior cingulate in the ASD group while the control group showed negative functional connectivity between these regions. The anterior cingulate is a key region in the attention network for cognitive control (Corbetta et al., 2008, Fan et al., 2012). Reduced functional activation of the anterior cingulate has been

associated with poor behavioural performance in executive control of attention using an attention orienting paradigm (Fan et al., 2012) therefore it is possible that the stronger connections to the anterior cingulate in the ASD group represent a compensatory mechanism which allows this group to perform the attention orienting task as well as controls. The ASD group also show stronger functional connectivity between ROIs in the VAN and several frontal regions; particularly the middle and inferior frontal gyri, brain regions that have also been implicated in cognitive control. This stronger positive functional connectivity between the VAN and various brain regions suggests that individuals with ASD may use an alternative neural mechanism to achieve successful attention orienting and reorienting.

3.4.4.2 Functional Connectivity during Valid Trials

During valid trials, there was aberrant connectivity between VAN ROIs and parietal, insular and inferior frontal regions in the ASD group. Relative to controls, the ASD group showed reduced strength of negative functional connectivity between the right superior and inferior parietal lobule regions and all selected ROIs (with the exception of the right precuneus). This indicates that there is less functional suppression between the right superior and inferior parietal lobules and the VAN in individuals with ASD. It is thought that the right superior and inferior parietal lobules play a role in regulating the selective modulation of the VAN (Corbetta et al., 2008). It may be that the ASD group requires a stronger input from parietal regions in order to modulate VAN successfully.

Overall the findings of this study suggest that the ASD group establish stronger functional connections between the VAN and multiple brain regions, which allow this

group to achieve exogenous attention orienting comparable to neurotypical controls. As discussed above, the ASD group showed reduced functional connectivity in the DAN, yet achieved normal behavioural performance. The DAN and VAN do not function independently (Corbetta et al., 2008), and it may be that stronger connectivity in the VAN provides a compensatory neural mechanism, which allows the ASD group to achieve normal endogenous attention orienting in this study.

3.4.5 Contribution of Findings to ASD Research

Attention orienting is of particular interest in ASD, as orienting of attention is a basic cognitive component necessary for joint attention (Leekam and Ramsden, 2006, Dawson et al., 2004), the ability to coordinate attention between an object and a person in a social context (McArthur and Adamson, 1996). Deficits in joint attention are one of the earliest signs of ASD (Murray et al., 2009, Whalen et al., 2006, Charman, 2003, Leekam et al., 2000, Dawson et al., 2004, Osterling and Dawson, 1994) and establishment of joint attention is required for normal socio-emotional development (Alessandri et al., 2005, Harris et al., 1999, Mundy et al., 1990). It is plausible that abnormalities in the basic cognitive function of attention orienting may contribute to joint attention and social communication deficits in ASD. A greater knowledge of the pathophysiology of abnormal attention orienting in ASD may lead to a greater understanding of the neurobiological underpinnings of impaired joint attention and social communication deficits in ASD. Future functional connectivity analyses may possibly incorporate a social component in the attention orienting paradigm to investigate the link between impaired attention orienting and joint attention.

3.4.6 Limitations

There were a number of limitations to this study. Participants with ASD were limited to male, right-handed individuals with average/above-average IQ. Therefore, results are specific to this group and not necessarily representative for all individuals on the spectrum. No external method such as eye-tracking was used to monitor participants' attention to the task. PPI connectivity analysis does not measure effective connectivity thus no causal relationships between brain regions can be identified. Furthermore, the study does not attempt to link functional abnormalities with structural brain abnormalities, an approach that is vital to increase the understanding of the pathophysiology of ASD. A detailed review of neuroanatomical abnormalities in ASD is outside the scope of this chapter; however, structural abnormalities have previously been reported in all brain regions that showed reduced activity in the ASD group in this study.

3.4.7 Conclusion

This study has demonstrated for the first time that functional connectivity in the dorsal and ventral attention networks is abnormal in an ASD population. The findings of this work have several important implications in terms of ASD research. Firstly, the significant abnormalities in functional connectivity in the ASD group lend strength to the theory of abnormal cortical connectivity in ASD. Secondly, the study provides important information about the alternative neural mechanisms that individuals with ASD may use to orient their attention. In the VAN, the ASD group engaged external regions such as the anterior cingulate to promote cognitive processing and facilitate intact attention orienting. Finally, the results suggest that individuals with ASD have

the ability to adjust neural mechanisms by strengthening functional connectivity between certain brain regions in order to maintain neurotypical cognitive function. The results of this study are exciting as they provide new insights into the complex neural mechanisms underpinning attention orienting in ASD.

4 Diffusion Magnetic Resonance Tensor Imaging Tractography of the Superior Longitudinal Fasciculus

4.1 Introduction

4.1.1 Overview of Diffusion Magnetic Resonance Imaging

As described previously in Section 1.5.3.2 and 2.4.3, diffusion magnetic resonance imaging (MRI) can be used to investigate the architecture of white matter tracts in vivo (Catani et al., 2002, Mori et al., 2002, Wakana et al., 2004, Catani and Thiebaut de Schotten, 2008, Ciccarelli et al., 2008, Basser et al., 2000, Jones et al., 1999). Diffusion MRI is based on the measurement of water molecular diffusion and is highly sensitive to subtle changes in white matter microstructure. If water movement is unrestricted by cellular barriers, diffusion will occur equally in all directions, known as isotropic diffusion. Conversely, if the passage of water molecules is impeded by barriers such as cell membranes or myelin sheaths which are present throughout white matter tissue, diffusion becomes anisotropic i.e. does not occur equally in all directions (Beaulieu, 2002).

4.1.2 Diffusion Tensor Imaging Tractography

Until recent years, whole brain voxel-based analyses were the main approach to investigate white matter differences using diffusion MRI data in ASD (Keller et al., 2007, Thakkar et al., 2008, Alexander et al., 2007a, Barnea-Goraly et al., 2004, Ke et al., 2009, Cheung et al., 2009, Shukla et al., 2010) however these studies do not provide

tract-specific microstructural information. The advent of Diffusion Tensor Imaging (DTI) tractography was exciting, as it allowed researchers to reconstruct white matter fibre bundles based on their dominant diffusion orientation (Catani et al., 2002, Mori et al., 2002, Basser et al., 2000). Furthermore, with the emergence of the theory of disrupted cortical connectivity, which hypothesises that the behavioural features characteristic of ASD are driven by disrupted functional and structural neural integration (Just et al., 2007, Just et al., 2004, Courchesne and Pierce, 2005a, Koshino et al., 2005, Koshino et al., 2008, Kana et al., 2006, Kana et al., 2009, Kana et al., 2007), ROI DTI tractography provided a reliable approach to investigate structural connectivity between specified brain regions in ASD.

The diffusion tensor model is the most commonly used methodological approach for analysis of diffusion MRI data. The diffusion tensor model can be used to determine the magnitude, orientation and anisotropic properties of white matter tissue (Alexander et al., 2007b). Fractional anisotropy (FA) is the most commonly used measure of white matter microstructural organisation and is reflective of changes in axonal density and diameter, myelination and fiber tract direction coherence (Beaulieu, 2002). An FA value of 0 indicates that diffusion is isotropic (equally distributed in all directions) and an FA value of 1 indicates that diffusion is anisotropic (occurs solely in one direction). A higher FA value reflects greater myelination of axons and stronger structural connectivity between neural regions (Vissers et al., 2012).

4.1.3 Constrained Spherical Deconvolution Based Tractography

The number of voxels in the brain containing crossing fibers has been estimated at approximately 33% (Behrens et al., 2007) however more recently, crossing and/or

kissing fibers are thought to be present in 90% of voxels in the brain (Jeurissen et al., 2013). Crossing fiber pathways and proximal tracts contribute to partial volume effects which are known to influence diffusion derived metrics (Basser et al., 2000, Jones, 2003, Alexander et al., 2002) and can hinder 'true' interpretation of DTI data (Vos et al., 2011). The neural architecture of the SLF is complex and involves numerous crossing and kissing fibers and standard DTI methods cannot accurately model the tensor in brain regions where such fibers exist. Constrained spherical deconvolution (CSD) based tractography can address the methodological difficulties associated with multiple fiber orientations and the diffusion tensor model (Jeurissen et al., 2011, Tournier et al., 2007). CSD tractography can manage partial volume effects and provide reliable diffusivity estimates by facilitating fiber tracking through complex neural regions (Tournier et al., 2008) thus generating more robust tract reconstructions.

Due to the presence of multiple fiber orientations, previous studies which have used diffusion metrics such as FA derived from tensor model estimates of eigenvalues may not have characterised white matter microstructural organisation adequately (Vos et al., 2012, Jones and Cercignani, 2010, Jeurissen et al., 2013, Wheeler-Kingshott and Cercignani, 2009). For example, if two fiber populations are crossing at 90^{o}, FA will be lower relative to FA in a single fiber population. However if one of the two crossing fibers populations decreases, for example due to a clinical pathology, FA will increase in this neural region (McGrath et al., 2013a). Therefore, an increase in FA may also represent a reduction in neural architecture complexity in clinical groups. Consequently, DTI studies demonstrate sensitivity to disrupted white matter organisation but cannot be specific about the type of change. With the implementation of CSD tractography, the diffusion measures extracted from the SLF

tract in this study may provide a more accurate measure of white matter microstructural organisation structure.

4.1.4 Abnormal White Matter Identified in Previous ASD Tractography Studies

Using ROI DTI tractography methods, abnormal white matter structural connectivity has been demonstrated in ASD in a number of major white matter intrahemispheric association tracts. The inferior frontal occipital fasciculus (McGrath et al., 2013a, Kumar et al., 2010, Chang et al., 2014), arcuate fasciculus (Fletcher et al., 2010, Kumar et al., 2010, Lo et al., 2011, Roberts et al., 2014), inferior longitudinal fasciculus (Chang et al., 2014, Wolff et al., 2012), uncinate fasciculus (Kumar et al., 2010, Lo et al., 2011), superior longitudinal fasciculus (Nagae et al., 2012, Verhoeven et al., 2012), cingulum (Kumar et al., 2010, Lo et al., 2011, Weinstein et al., 2011a, Ikuta et al., 2014) and the corpus callosum (Hong et al., 2011, Weinstein et al., 2011a, Kumar et al., 2010) have all demonstrated disrupted white matter organisation. Some of the studies have reported increased FA in ASD relative to controls (Weinstein et al., 2011a, Wolff et al., 2012) while others report reduced FA across white matter tracts (Kumar et al., 2010, Lo et al., 2011, McGrath et al., 2013a, Chang et al., 2014, Ikuta et al., 2014). Given that white matter development changes across the lifespan (Lebel et al., 2008), the varied age groups examined in these studies are likely to have influenced results. Some studies explored white matter differences in adolescence (aged between 12 and 18 years) (Lo et al., 2011, Fletcher et al., 2010, Ikuta et al., 2014, McGrath et al., 2013a) while others examined children (aged between 6 and 12 years) with ASD (Wolff et al., 2012, Weinstein et al., 2011a, Kumar et al., 2010, Hong et al., 2011). However, even

within studies of the same age group, results differ. For example, in adolescents, Fletcher et al. (2010) found white matter differences in the arcuate fasciculus while another study which employed constrained spherical deconvolution (CSD) tractography (an approach developed to overcome the limitations of DTI) did not (McGrath et al., 2013a) thus highlighting the importance of technical considerations when interpreting results. Furthermore, Lebel et al. (2008) demonstrated that the developmental trajectory of white matter differs across tracts in neurotypical individuals thus highlighting the value of tract specific studies of white matter architecture in ASD.

4.1.5 Abnormal White Matter Organisation of Tracts Associated with Behavioural and Executive Function Deficits in ASD

ROI tractography studies suggest that disrupted white matter organisation is associated with behavioural deficits characteristic of ASD. Reduced FA in the corpus callosum (forceps major) was inversely correlated with higher ADOS communication subscale scores (Hanaie et al., 2014). Similarly, Catani et al. (2008) found that increased severity of social impairment as measured by the ADI-R was associated with decreased FA values in the left superior cerebellar peduncle. Additionally, research suggests that aberrant structural connectivity is also related to executive dysfunction in ASD. McGrath et al. (2013a) demonstrated that reduced FA in the right inferior frontal-occipital fasciculus (IFOF) was associated with atypical visuospatial processing in ASD while another study found that reduced FA in the cingulum was correlated with higher scores on the behaviour rating inventory of executive function (BRIEF) driven primarily by reduced set-shifting ability (Ikuta et al., 2014). Furthermore, increased FA

values were correlated with better inhibitory control in an extracted putamen tract (Langen et al., 2012). Given the role of fronto-posterior cortical development in cognitive, language, social and emotional processing (Stuss and Knight, 2002) and the deficits exhibited in these domains in ASD [see (Schipul et al., 2011) for review], the current study focuses on examination of a major fronto-parietal white matter tract, the superior longitudinal fasciculus (SLF). In light of previous studies, it can be postulated that anomalies of white matter microstructure within the SLF may underpin behavioural and executive function deficits in ASD.

4.1.6 Superior Longitudinal Fasciculus Development in Neurotypicals

The superior longitudinal fasciculus (SLF) is a bilateral white matter association fibre tract. Association tracts are fiber pathways which connect neural regions within a cerebral hemisphere. The SLF connects the parietal and temporal brain regions with the frontal lobes (Schmahmann et al., 2008). Observations of white matter maturation have consistently demonstrated that fractional anisotropy increases bilaterally with age in the superior longitudinal fasciculus (Lebel et al., 2008, Peters et al., 2012, Snook et al., 2007, Bava et al., 2010, Asato et al., 2010, Giorgio et al., 2008, Qiu et al., 2008) and 90% of the maximum FA value is attained between 13 and 20 years of age (Asato et al., 2010, Lebel et al., 2008). Researchers have hypothesised that the increased structural connectivity, which occurs in frontal white matter projections during adolescence, may represent the development of higher order cognitive processes and executive functioning (Liston et al., 2006, Luna et al., 2004, Geier and Luna, 2009, Ashtari et al., 2007).

4.1.7 Structure of the Superior Longitudinal Fasciculus

Previous primate studies (Petrides and Pandya, 1984, Schmahmann et al., 2007, Schmahmann and Pandya, 2006, Pandya and Seltzer, 1982, Seltzer and Pandya, 1980) and post-mortem histological findings (Martino et al., 2011, Thiebaut de Schotten et al., 2011) have provided crucial anatomical information regarding the projections of the SLF and since the advent of diffusion tractography, researchers have been applying this method to try and elucidate the structure of the SLF in humans. It has been established that the superior longitudinal fasciculus is a bilateral white matter association tract largely composed of three fronto-parietal longitudinal pathways, the SLF I, II and III (Thiebaut de Schotten et al., 2012, Kamali et al., 2014, Schmahmann et al., 2008). The SLF I is the most dorsal pathway and extends from the precuneus and superior parietal lobule (Brodmann areas, BA 4, 5 and 7) to the rostral superior frontal gyri, premotor and anterior cingulate cortices (Brodmann areas, BA 6, 8, 9 and 32). The SLF II projects from the anterior intermediate parietal sulcus and the angular gyrus (Brodmann areas, BA 39 and 40) to the posterior superior and middle frontal gyri / dorsolateral prefrontal cortex (Brodmann areas, BA 6, 8, 9 and 46). The SLF III is the most ventral pathway, and connects the temporoparietal junction / supramarginal gyrus (Brodmann area, BA 40) to the inferior frontal gyrus and prefrontal cortex (Brodmann areas, BA 6, 44, 45 and 47). A fourth and fifth component of the SLF have also been described. The SLF IV, commonly known as the arcuate fasciculus, connects the superior temporal gyrus and the ventrolateral prefrontal cortex and the fifth component, namely the temporoparietal SLF, is believed to connect the temporal and parietal lobes (Kamali and Hasan, 2014).

4.1.8 Function of the Superior Longitudinal Fasciculus

The majority of studies probing the function of the SLF in have focused on the branches of the tract as a whole. Research has established a role for the SLF in language processing (Glasser and Rilling, 2008, Bernal and Altman, 2010, Gold et al., 2007, Brauer et al., 2011, Ashtari et al., 2007) however the function of the SLF in other cognitive processes is less clear. Some studies have indicated that increased FA in the left SLF is associated with greater spatial working memory performance (Vestergaard et al., 2011, Burzynska et al., 2011) while Urger et al. (2014) found that higher FA values bilaterally in the SLF were associated with better attention and set-shifting capabilities. This study also illustrated an association between white matter organisation and inhibitory control in the left SLF. The importance of the SLF in attention function has also been suggested from the results of research by Ge et al. (2013) who found that FA values in the right SLF were positively correlated with attention orienting performance, and from the work of Chaddock Heyman et al. and Noble et al. who have demonstrated that increased cognitive control was associated with higher FA values in the SLF (Noble et al., 2013, Chaddock-Heyman et al., 2013). Collectively, these studies demonstrate that the SLF as a whole subserves a multitude of complex cognitive processes.

4.1.9 Function of the Superior Longitudinal Fasciculus Branches

Very few studies have investigated the discrete functions of each branch of the SLF. Research by Thiebaut de Schotten et al. (2011) suggested that the SLF I links the functionally connected neural regions within the dorsal attention network (DAN). This work also indicated that the SLF II connected the prefrontal region within the DAN to

the parietal region with the ventral attention network (VAN) facilitating communication between these two attentional networks. The SLF III was reported to link brain regions solely within the VAN. The VAN is considered to be a right hemisphere dominant functional network while the DAN is a bilateral attention network (Corbetta and Shulman, 2002). White matter organisation of the SLF branches followed a similar anatomical pattern; the SLF I was shown to be symmetrical across hemispheres while the SLF III demonstrated an anatomical preference for the right hemisphere. The SLF II indicated a trend towards right hemisphere dominance but this appeared dependent on behavioural characteristics. The study showed that larger right SLF II volumes correlated with enhanced visuospatial processing and postulated that anatomical asymmetry may predict behavioural performance (Thiebaut de Schotten et al., 2011). Other studies have hypothesised that the SLF I regulates higher aspects of motor behaviour while the SLF II and SLF III are involved in visuo-spatial perception, attention and higher order somatosensory input respectively (Makris et al., 2005, Schmahmann and Pandya, 2006, Schmahmann et al., 2007).

4.1.10 White Matter Differences in the Superior Longitudinal Fasciculus in ASD

A number of whole brain voxel-wise DTI studies have indicated abnormal white matter microstructure in the SLF in ASD (Cheung et al., 2009, Weinstein et al., 2011a, Shukla et al., 2011, Groen et al., 2011, Noriuchi et al., 2010) however the results of these studies have been quite varied. Some studies have reported increased FA in the SLF (Cheung et al., 2009, Weinstein et al., 2011a) while others found a reduction in FA in the same tract (Noriuchi et al., 2010, Groen et al., 2011, Shukla et al., 2011). These

differences are likely to have arisen from lack of power (n < 18) (Cheung et al., 2009, Groen et al., 2011, Noriuchi et al., 2010) and methodological heterogeneity; some adopted a standard voxel-based approach (Cheung et al., 2009, Groen et al., 2011), while others used an alternative approach called Tract Based Spatial Statistics (TBSS) (Shukla et al., 2011, Weinstein et al., 2011a). Only two previous studies have used DTI-tractography to isolate the SLF in ASD (Nagae et al., 2012, Verhoeven et al., 2012) but neither of these investigated the individual branches of the SLF. Nagae et al. (2012) found that structural connectivity in the left SLF was disrupted in individuals with ASD with a co-morbid language impairment but not in the children with ASD without a language difficulty. Similarly, Verhoeven et al. (2012) found no difference in structural connectivity in ASD (specifically without language impairment) relative to controls. These studies suggest that aberrant white matter organisation in the SLF is related to language impairment rather than ASD symptomology alone.

From the current literature, it is apparent that the discrete branches of the SLF have never been isolated in an ASD population. This is an important avenue of research to pursue in ASD due to role of the SLF in the function of attention. Impaired attention has been consistently reported in ASD (Belmonte and Yurgelun-Todd, 2003, Dawson et al., 2004, Elsabbagh et al., 2013, Keehn et al., 2010). It has been suggested that different branches of the SLF appear to play distinct roles in attention (Thiebaut de Schotten et al., 2011) thus investigating each branch in isolation is important for understanding the neural correlates of attention dysfunction in ASD. Furthermore, the process of attention is lateralised (Corbetta et al., 2008, Corbetta and Shulman, 2002) and in a neurotypical population, FA was also found to be lateralised in the branches of the SLF (Thiebaut de Schotten et al., 2011). Therefore, investigation of SLF branch

symmetry in an ASD population may also contribute to our understanding of attention deficits characteristic of the disorder. Some studies have reported associations between disrupted structural integrity of the SLF and clinical characteristics but the findings have been heterogeneous (Bakhtiari et al., 2012, Gibbard et al., 2013) thus isolating the branches of the SLF may aid clarification of these inconsistent results. With advanced imaging software, it is now possible to perform in-depth investigations of the white matter organisation within fiber pathways. Evaluating diffusion measures of white matter in segments along the tract may also identify regions of abnormality that may be overlooked due to averaging diffusion metric values along the whole tract.

4.1.11 Aims

The main objectives of this study are to:

1. Use CSD based tractography to isolate the bilateral SLF I, II and III and investigate diffusion measures of fractional anisotropy (FA), linear diffusion coefficient (CL) and and planar diffusion coefficient (CP) in ASD.
2. Evaluate asymmetry for each tract and diffusion metric across the left and right hemisphere between groups.
3. Determine if diffusion measures that differ between groups are associated with social and communication deficits and / or restricted repetitive behaviours in ASD.
4. Perform tract segmentation analysis to evaluate if diffusion measures differ in particular regions of the white matter bundle.

4.2 Methods

This section describes the study population, the diffusion MRI preprocessing pipeline and the CSD based tractography protocol used to isolate the branches of the SLF. A description of the diffusion measures and the statistical analysis approach used is also outlined in the following paragraphs.

4.2.1 Participants

Participants included in this analysis were taken from the 'McGrath' and 'Fitzgerald' samples. Details of the participants for this study (study 2) are described in Table 4.1 and in Chapter 2, Section 2.2. Distribution of social and communication deficits and restricted repetitive behaviours are illustrated in Figures 4.1 and 4.2 respectively. Tests of normal distribution of these behavioural measures are reported in Table 4.2.

Table 4.1. Participant Demographics

	Control	**ASD**	**P-value**
Number	45	45	
Gender	Male	Male	
Mean age ± SD; range	16.55 ± 3.04; 10.25-24.2	15.91 ± 3.3; 10-24	0.336
Mean Full Scale IQ ± SD; range	115 ± 14.62; 81-147	109.5 ± 15.9; 78-145	0.098
Social Communication Deficits		24.29 ± 6.82: 12-40	
Restricted Repetitive Behaviours		4.87 ± 2.53: 0-12	
ADOS: Social and Communication		9.82 ± 2.871; 7-19	

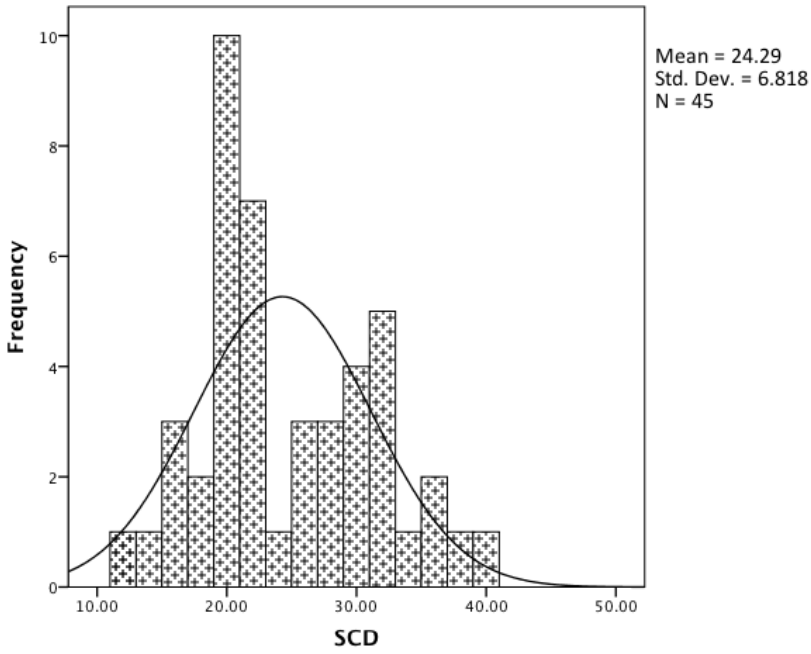

Figure 4.1. Distribution of Social and Communication Deficits Domain Scores

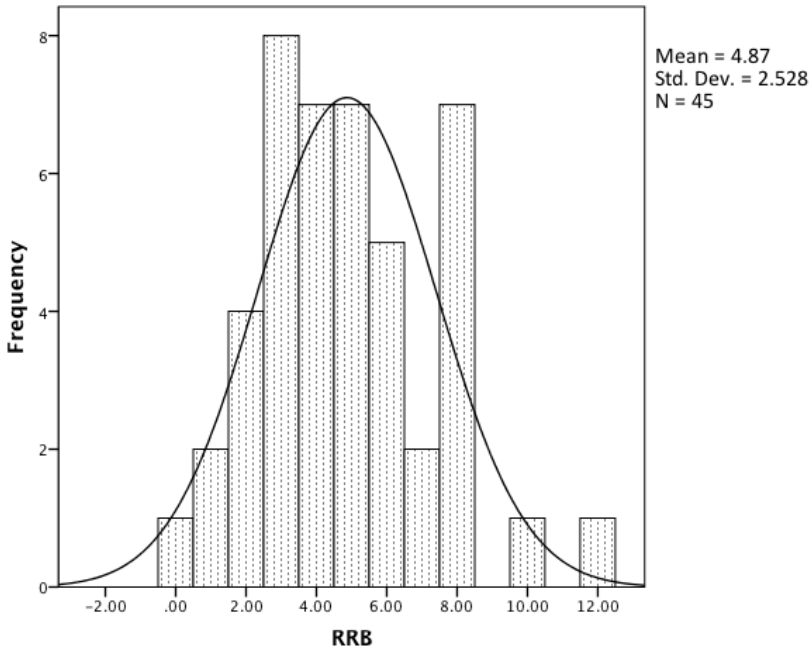

Figure 4.2. Distribution of Restricted and Repetitive Behaviours Domain Scores

Table 4.2. Test of Normal Distribution for Behavioural Measures

Behavioural Measure	Shapiro-Wilk Statistic	Shapiro-Wilk P-Value	Skew	Kurtosis
Social and Communication Deficits	0.979	0.365	0.392	-0.699
Restricted and Repetitive Behaviours	0.966	0.78	0.528	0.22

4.2.2 Diffusion-Weighted Imaging Preprocessing

High angular resolution diffusion imaging (HARDI) data was acquired in a 3T Philips Scanner in 61 isotropic directions with a b-value = 1500 s/mm^2 (see Chapter 2, Section 2.5.3 for details of acquisition protocol). Preprocessing of diffusion data was completed using ExploreDTI software v4.8.3 (http://www.ExploreDTI.com) (Leemans et al., 2009). Firstly, raw diffusion MRI files were converted to matlab files. During this conversion, the tensor model was applied to the data using the robust estimation of tensors by outlier rejection (RESTORE) method, which acts to improve tensor estimation by correcting for image distortions including subject motion, cardiac pulsation and fat suppression (Chang et al., 2005). Each dataset was visually inspected to ensure orientation of gradient components was maintained during the conversion. A rectangular 'gate' or region of interest (ROI) was drawn around the vicinity of corpus callosum on the axial, sagittal and coronal planes and 'glyphs' were drawn to ensure that the widely used colour convention was correct (Red = left - right, Green = anterior – posterior, Blue = dorsal – ventral) and the gradient components had not flipped. If any of the components were compromised during this process, the conversion was

repeated once the sign (+ or -) of the flipped gradient component was adjusted in the parameter set-up. Data quality checks were then performed. Diffusion weighted images (DWI) were looped and visually inspected in addition to eye-balling of residual and outlier profiles to assess the presence of gross artifacts. Subject motion and eddy current induced geometric distortions are the most frequently detected artifacts in diffusion weighted data (Soares et al., 2013) and more recently, the issue of echo-planar imaging (EPI) geometric and intensity distortion for DTI tractography has also been highlighted (Irfanoglu et al., 2012). Each of these distortions was corrected for in one interpolation step to minimise blurring effects. B-matrix reorientation was also performed within this single step to adjust for any rotational differences that may occur during data correction (Leemans and Jones, 2009). The tensor estimation for native data correction was set to 4 to equate to the RESTORE method. For the subject motion and eddy current correction, the number of iterations was set at 2000 and the number of resolutions was set to 4 . Each dataset was realigned to the non-diffusion weighted B0 image applying a full 12 DOF affine transformation and cubic interpolation. The default settings do not include correction for EPI distortions so this was selected during the parameter set-up. The diffusion-weighted images were resampled to an undistorted T1 image to unwarp EPI deformations in the data. For this resampling, the number of iterations was set at 2000 and the number of resolutions was set to 4. All other parameters remained at the recommended default setting (Leemans A., DTI Workshop, Rotterdam, 2013).

4.2.3 Constrained Spherical Deconvolution Based Tractography

As previously described in Section 4.1.3, constrained spherical deconvolution (CSD) is a methodological approach developed to overcome issues in tractography analyses due to voxels containing multiple fiber orientations (Tournier et al., 2007). The branches of the SLF were isolated using CSD tractography (Jeurissen et al., 2011). This algorithm involves obtaining the fiber orientation distribution (FOD) from the diffusion-weighted signal while preserving the angular resolution and remaining robust to artifacts due to noise. Selection of a seed point begins fiber tracking and the diffusion-weighted signal at that point is extracted using tri-linear interpolation. Fiber orientation distribution is estimated across the whole-brain in a grid of $2mm^3$ segments. Determination of tract direction in each 2mm segment is defined by the fiber orientation within the closest segment that precedes it. The trajectory of the tract continues along the specified direction in a fixed step size of 1mm. Fiber tracking ceases when FOD peak intensities are beneath a fixed threshold of 0.2, the maximum angle of 30^o is exceeded or the tract extends outside of a specified brain mask.

4.2.4 Superior Longitudinal Fasciculus I, II and III Extraction Protocol

The protocol for extracting the SLF I, II and III was based on Thiebaut de Schotten's work on visuospatial attention (Thiebaut de Schotten et al., 2011). Isolation of the three branches of the SLF was performed using atlas-based tractography, an automated approach for tract extraction validated by Lebel et al. (2008). This automated procedure is robust to spatial normalisation errors as fractional anisotropy thresholds used for tractography aid spurious fiber elimination in addition to enlarging

seed and target regions to accommodate misaligned structures. A representative single subject was selected for the basis of the atlas or template. On the coronal slice, a large 'AND' gate was drawn at the posterior commissure line to capture tracts passing through the parietal lobe. An 'AND' gate is a region of interest (ROI) which incorporates any fibers passing through. On the axial plane, a large 'NOT' gate was drawn along the anterior/posterior commissure line at the temporal lobe to eliminate fibers passing through this region, specifically the SLF IV otherwise known as the arcuate fasciculus. A 'NOT' gate is a region of interest (ROI) which excludes any fibers passing through. On the coronal plane, individual 'AND' gates were drawn at the anterior commissure line in the frontal lobe specific to the branch of the SLF to be isolated (Figure 4.3). To extract the SLF I, an 'AND' gate was drawn around the white matter of the superior frontal gyrus on the coronal axis at the anterior commissure line. To isolate the SLF II, an 'AND gate was drawn around the white matter of the middle frontal gyrus on the coronal axis along the anterior commissure line and finally, the SLF III was extracted by drawing an 'AND' gate around the white matter of the inferior frontal/precentral gyrus on the coronal axis at the anterior commissure line. The tractography algorithm extracted all fibers which passed through the specified 'AND' gates and eliminated fibers which passed through the temporal 'NOT' gate to produce the SLF I, II and III. Individual tracts were visually inspected for each subject and non-blinded editing of the isolated tracts was performed where necessary. In the event that a tract did not appear to have been isolated as expected (see Figure 4.4), the warped templates and ROIs for that subject (which are automatically produced during atlas-based tractography) were visualised to determine which ROI(s) did not warp correctly. These ROI(s) were re-drawn and the compromised tract was isolated

manually. Based on the tract information generated, an average tract length was set for every subject for each tract. The tracts were then recalculated in order to remove extensive spurious tracts. Subsequent 'NOT' gates were drawn if necessary to remove any remaining spurious tracts not accounted for in the previous step.

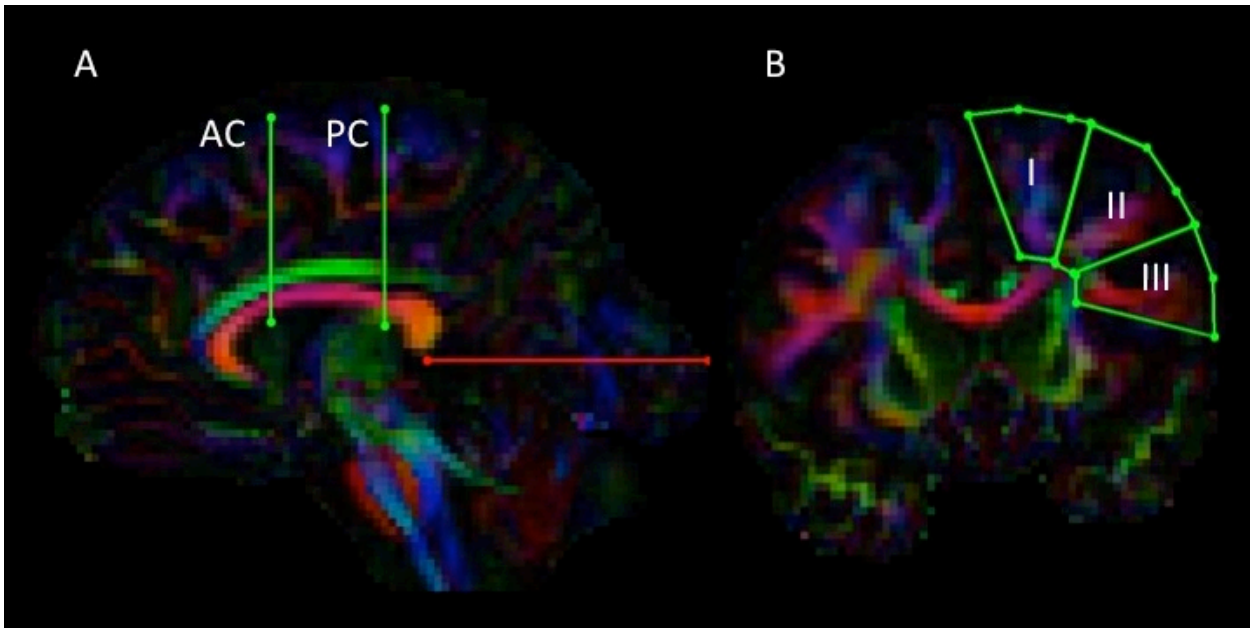

Figure 4.3. Representation of the Protocol Used to Extract Three Branches of the Superior Longitudinal Fasciculus (SLF).

(A) 'AND' gates were drawn at the anterior commissure (AC) and posterior commissure (PC) on the coronal axis and a 'NOT' gate was drawn on the axial plane. One large 'AND' gate was drawn at the PC line. **(B)** Three individual 'AND' gates were drawn at the AC line for each branch of the SLF (I, II or III). Images are displayed in radiological convention (left is right).

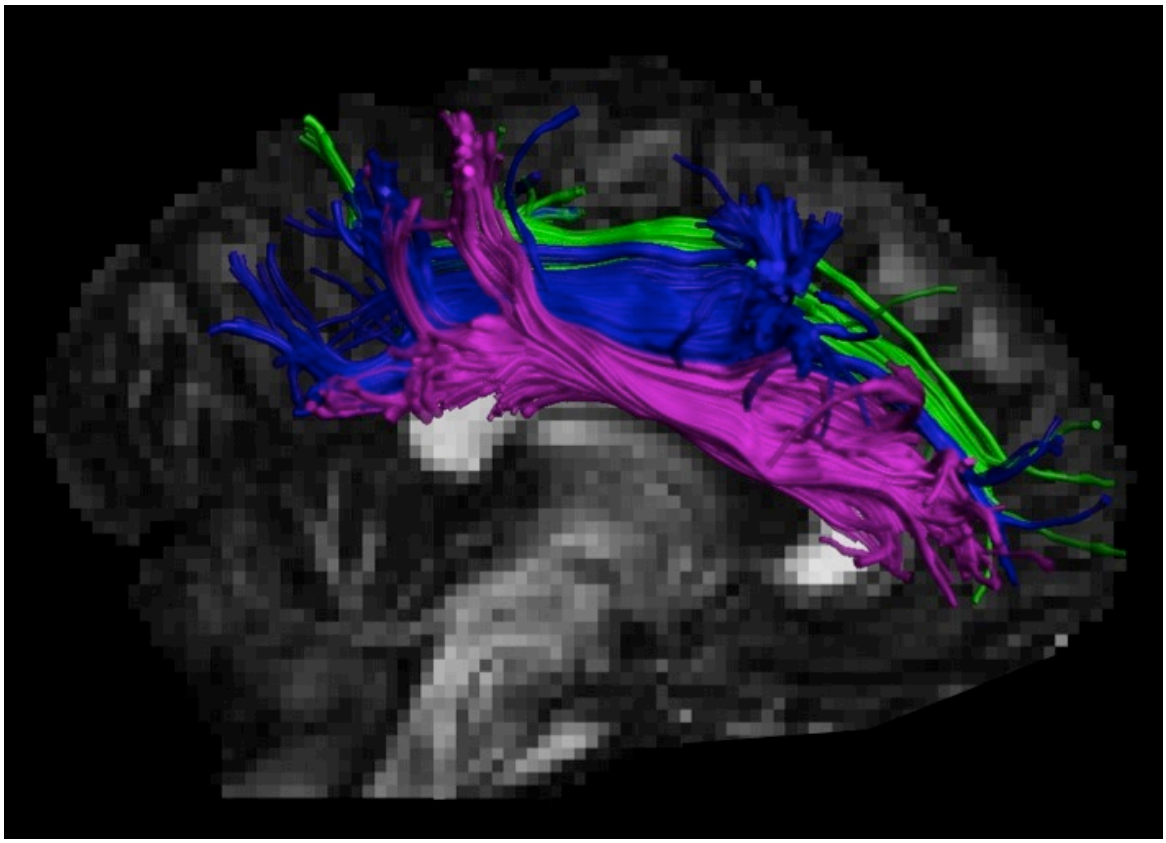

Figure 4.4. Representation of the Right Superior Longitudinal Fasciculus Subdivided into Three Branches.

Branches are illustrated in three colours; SLF I (Green), II (Blue) and III (Purple) from a single subject.

4.2.5 Measures of White Matter Microstructure

Once all of the tracts were isolated (Figure 4.4), diffusion metrics from each branch of the SLF for every individual were extracted. The diffusion metrics examined were fractional anisotropy (FA), linear diffusion coefficient (CL) and planar diffusion coefficient (CP). Fractional anisotropy is the most commonly reported measure in DTI studies and is thought to reflect white matter microstructure in terms of myelination, axonal organisation and fiber density. Fractional anisotropy is calculated based on

three eigenvalues; the first eigenvalue (λ_1) represents parallel or axial diffusivity (AD), and the second and third eigenvalues (λ_2 and λ_3) which an average of illustrates perpendicular or radial diffusivity (RD) (Alexander et al., 2007b) (see Chapter 2, Section 2.4.3 for more details). Perpendicular diffusivity is believed to represent myelination of axons while parallel diffusivity is thought to reflect axonal degeneration (Song et al., 2002). Linear and planar diffusion coefficients are also based on eigenvalues but also incorporate geometric information of the diffusion tensor hence providing more interpretable information relating to abnormalities in white matter architecture in comparison to FA (Westin et al., 2002). CL is closely related to axial diffusivity while CP is closely related to radial diffusivity. Given that these metrics offer more representative information and reflect parallel and perpendicular diffusion, they were used in addition to FA to investigate white matter organisation in the SLF. A high CL value indicates a singular dominant fiber orientation in a voxel while a high CP value suggests a number of crossing fibers in a voxel (Vos et al., 2012).

4.2.6 Statistical Analyses

IBM SPSS Statistics, Version 19 (Armonk, NY: IBM Corp) was used to investigate between-group differences in white matter microstructure. Firstly, all diffusion measures were inspected to determine the presence of outliers for each group. Outliers were removed to ensure all data was within the 95% confidence interval range prior to statistical analyses. Multivariate analyses were performed to examine between-group (ASD and control) differences on measures of FA, CL and CP in each branch of the SLF bilaterally. A lateralisation index (LI) was also calculated for each metric to provide an estimate of hemispheric asymmetry for each subject using the

equation (Right – Left)/(Right + Left) [see (Thiebaut de Schotten et al., 2011) supplementary materials]. A negative LI value suggested greater leftward asymmetry while a positive LI estimate indicated a greater right asymmetry. Multivariate analyses were performed to examine between-group (ASD and control) differences on lateralisation indices for each measure of FA, CL and CP. All statistical analyses were Bonferroni corrected at a significance level of $p < 0.05/3 = 0.0166$.

4.2.7 Correlation Analyses

Exploratory bivariate Spearman correlations were performed to investigate the relationship between clinical characteristics of ASD and white matter microstructural differences in ASD. For all diffusion measures showing between group differences, correlation analysis was performed using two measures; an index of social communication and an index of restricted repetitive behaviours. Bonferroni correction of correlation analyses were performed based on the number of clinical measures used ($p = 0.05/2 = 0.025$).

4.2.8 Tract Segmentation Analyses

Standard tractography methods average the diffusion measures across tracts and this approach may not detect discrete abnormalities in white matter microstructure along the tract (Colby et al., 2012). In order to explore localised differences in organisation of white matter at the voxel level, tract segmentation analyses were performed on each branch of the SLF bilaterally. The average length of each tract across groups was obtained and divided by 2 to determine the number of divisions required to yield segments measuring approximately 2mm. It is recommended that segments are sub-divided based on the voxel size set during data acquisition (Leemans

A., DTI Workshop, Rotterdam, 2013). For example, the average length of the left and right SLF I in this dataset was 80mm thus both tracts were subdivided into 40 segments for each subject. The bilateral SLF II was subdivided into 41 segments, the right SLF III into 37 segments and the left SLF III into 41 segments for each individual. Diffusion metrics of FA, RD, and AD were extracted from each 2mm segment. In order to provide greater information about the possible neuropathology underlying changes in FA, AD and RD metrics were used as CL and CP metrics were not available for along tract statistical analyses. Multivariate analyses were performed examining between group (ASD and control) differences in the diffusion metrics at a significance threshold of $p < 0.05$. This was an exploratory analysis and correction for multiple comparisons was not carried out.

4.3 Results

4.3.1 Diffusion Metrics

In the left SLF I, the ASD group showed greater linear coefficient diffusivity (CL) ($F(1, 88) = 9.204$, $p = 0.003$, $\eta p^2 = 0.095$) and a strong trend towards greater fractional anisotropy (FA) ($F(1, 88) = 5.772$, $p = 0.018$, $\eta p^2 = 0.062$) in comparison to the control group. In the right SLF II, the ASD group also showed greater FA ($F(1, 88) = 7.221$, $p = 0.009$, $\eta p^2 = 0.076$) and greater CL ($F(1, 88) = 7.862$, $p = 0.006$, $\eta p^2 = 0.006$) relative to controls (Table 2).

4.3.2 Lateralisation Indices

In the SLF II, the ASD group had significantly greater right lateralisation of FA ($F(1, 88) = 8.792$, $p = 0.004$, $\eta p^2 = 0.091$) and borderline significance in CL ($F(1, 88) = 5.899$, $p = 0.017$, $\eta p^2 = 0.063$) in comparison to the control group. The SLF I and SLF III did not differ in term of lateralisation on any of the measures.

Table 4.3. Between-Group Differences in Diffusion Metrics

Tract	Hemisphere	ASD ± SD	Control ± SD	P-value
Fractional Anisotropy				
SLF I	Left	0.472±0.031	0.456±0.03	0.018
	Right	0.467±0.034	0.468±0.035	0.954
SLF II	Left	0.378±0.031	0.378±0.029	0.962
	Right	0.412±0.053	0.38±0.054	**0.009***
SLF III	Left	0.391±0.032	0.396±0.03	0.405
	Right	0.376±0.034	0.383±0.032	0.312
Planar Diffusion Coefficient (CP)				
SLF I	Left	0.241±0.03	0.246±0.03	0.419
	Right	0.248±0.033	0.248±0.032	0.973
SLF II	Left	0.196±0.02	0.203±0.02	0.174
	Right	0.208±0.028	0.208±0.025	0.955
SLF III	Left	0.233±0.032	0.231±0.029	0.679
	Right	0.221±0.04	0.222±0.03	0.828
Linear Diffusion Coefficient (CL)				
SLF I	Left	0.396±0.028	0.377±0.031	**0.003***
	Right	0.386+0.04	0.387+0.038	0.888
SLF II	Left	0.335±0.019	0.33±0.024	0.369
	Right	0.359±0.053	0.33±0.047	**0.006***
SLF III	Left	0.321±0.03	0.328±0.024	0.185
	Right	0.314±0.027	0.320±0.028	0.289

* Indicates significant between-group differences at $p < 0.0166$. SLF = Superior Longitudinal Fasciculus, SD = Standard Deviation

Table 4.4. Between-Group Differences in Lateralisation Indices for White Matter Diffusion Measures

Measure	ASD ± SD	Control ± SD	P-value
Fractional Anisotropy			
SLF I	-0.005±0.044	0.012±0.043	0.068
SLF II	0.0397±0.065	0.007±0.059	**0.004****
SLF III	-0.02±0.044	-0.018±0.05	0.807
Planar Diffusion Coefficient (CP)			
SLF I	0.0135±0.069	0.0029±0.081	0.508
SLF II	0.0275±0.08	0.013±0.079	0.388
SLF III	-0.0299±0.099	-0.0178±0.0946	0.555
Linear Diffusion Coefficient (CL)			
SLF I	-0.0146±0.054	0.0119±0.0599	0.031
SLF II	0.0309±0.075	-0.0045±0.062	0.017
SLF III	-0.0087±0.059	-0.0118±0.053	0.796

* Indicates significant between-group differences at $p < 0.0166$. SLF = Superior Longitudinal Fasciculus, SD = Standard Deviation

4.3.3 Correlation Analyses

There was no correlation between social and communication deficits or restricted repetitive behaviours and the white matter integrity measures that differed significantly between groups. Correlation analyses of the lateralisation indices in the

SLF II revealed an association between FA and social and communication deficits ($r = 0.301$, $p = 0.044$) indicating that greater rightward asymmetry of FA is correlated with greater social and communication deficits in ASD. However, given the number of correlations performed, these results should be interpreted with caution.

4.3.4 Tract Segmentation Analyses

Each branch of the SLF was segmented to investigate along-tract differences in FA, AD, RD and MD. Between-group differences in FA and AD were identified in a number of segments along the left SLF I and the right SLF II (Figure 3). Differences in radial diffusivity were also identified in a number of segments along the left SLF I and the right SLF II; these differences were obscured by the averaging method involved in the whole tract analysis.

The SLF I was subdivided into 40 2mm segments. In the ASD group relative to controls, greater FA was observed in segments 13 – 23, $p < 0.05$, greater AD was observed in segments 10,11, 16 – 30, $p < 0.05$ and reduced RD was observed in segments 17 – 20, $p < 0.05$.

Between group differences in radial diffusivity were also identified in a number of segments along the left SLF I and the right SLF II. The SLF II was fragmented into 41 2mm segments. In the ASD group, significantly greater FA was identified in the SLF II in segments 13 – 27 and 33 – 37, $p < 0.05$, greater AD was identified in segments 5 – 26, $p < 0.05$ and reduced RD was identified in segments 16-20, $p < 0.05$ relative to controls. No differences were found along the other tracts.

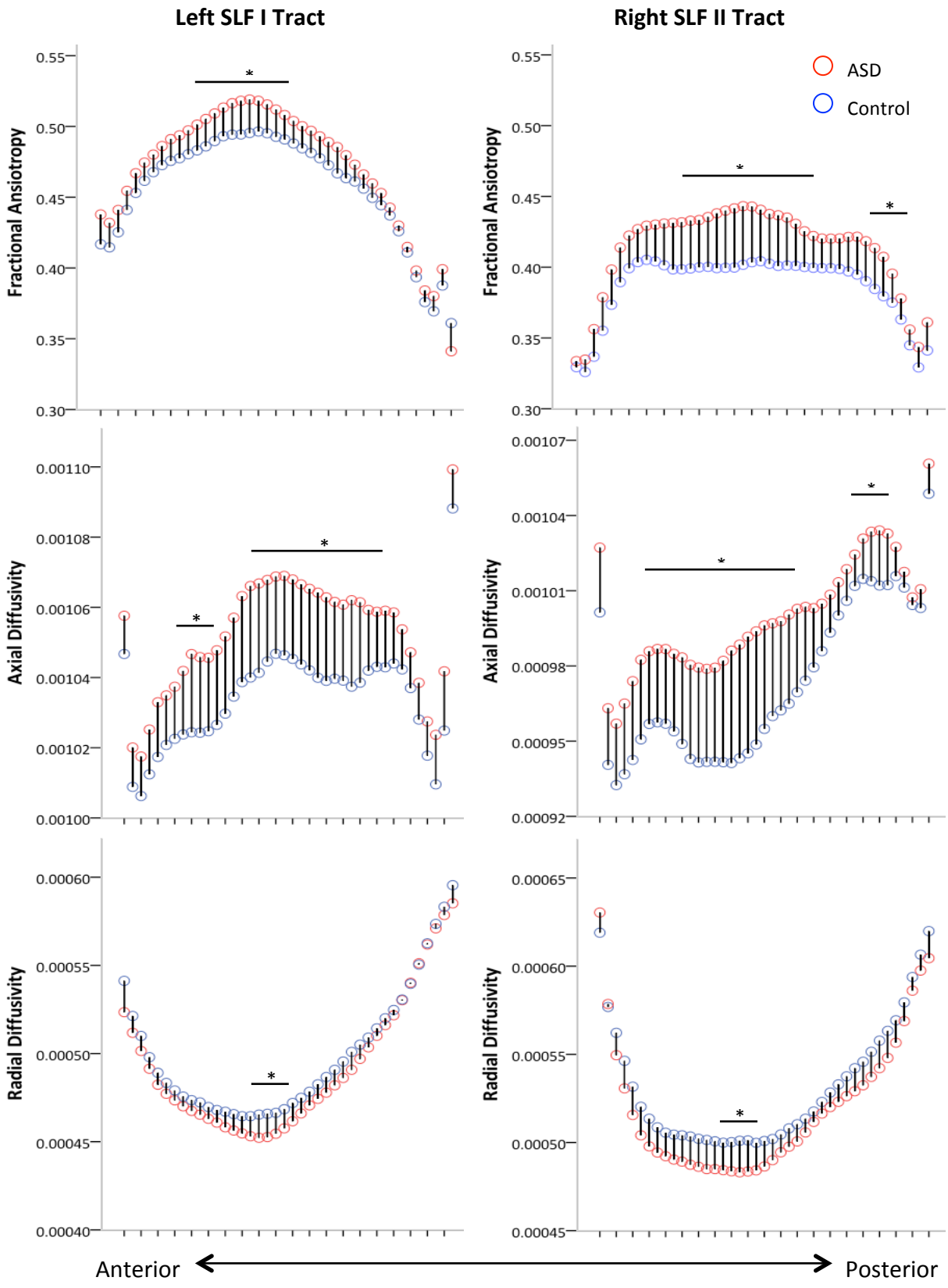

Figure 4.5. Tract Segmentation of the Left SLF I and the Right SLF II

Diffusion measures of fractional, axial and radial diffusivity are presented in the left SLF I and right SLF II, uncorrected at $p < 0.05$.

4.4 Discussion

4.4.1 Overview of Findings

This is the first study to use CSD-based tractography to investigate the organisation of white matter microstructure in three branches of the SLF in an ASD population. Results have shown that white matter organisation is disrupted in the left SLF I and the right SLF II in ASD. Furthermore, there was greater rightward asymmetry of FA in the ASD group relative to the control group. Exploratory along-tract segmentation analyses have demonstrated that there were certain regions that showed highly significant between-group differences in diffusion measures that would have driven the observed whole-tract differences in the left SLF I and right SLF II.

4.4.2 Discussion of Findings in Relation to Previous Literature

In both the left SLF I and right SLF II, greater FA and CL was observed in the ASD group relative to controls. Greater FA has been previously observed in an ASD cohort in white matter studies including young children (Weinstein et al., 2011a, Wolff et al., 2012, Billeci et al., 2012), older children (Cheung et al., 2009) and adolescents (Cheng et al., 2010) and greater FA specifically in the SLF has been reported in some of these studies (Cheung et al., 2009, Weinstein et al., 2011a, Cheng et al., 2010). The results from other studies provide a more mixed picture with reports of reduced FA across white matter tracts (Fletcher et al., 2010, Ikuta et al., 2014, Lo et al., 2011, McGrath et al., 2013b, Noriuchi et al., 2010, Groen et al., 2011) and three studies examining the SLF have reported reduced FA in this tract (Groen et al., 2011, Noriuchi et al., 2010, Shukla et al., 2011). Methodological differences between studies may have led to this

rather heterogeneous pattern of results. As outlined by Jones and Cercignani (2010), each processing step in MRI diffusion analysis is susceptible to bias and may lead to inaccurate inferences about the data. Therefore, it is important to note that different approaches such as VBM, TBSS and ROI tractography were used to derive the results of the studies cited above. Furthermore, it could be argued that previous studies which have described isolating the SLF using ROI tractography (Nagae et al., 2012, Verhoeven et al., 2012) may have extracted the fourth branch of the SLF, the arcuate fasciculus (AF). Nagae et al. (2012) found disrupted structural connectivity in the SLF reflected by increased MD in individuals with ASD with a co-morbid language impairment but not in the children with ASD without a language difficulty. Given the role of the AF in language processing, this may suggest that differences in FA were only observed when ASD was co-morbid with language impairment.

4.4.3 Disrupted Organisation of the Superior Longitudinal Fasciculus I in ASD

Aberrant structural connectivity was observed in the left SLF I, which projects between the precuneus and superior parietal lobule and the superior frontal gyrus and the anterior cingulate cortex. It has been established that the left superior frontal gyrus is involved in executive functions such as planning, cognitive flexibility (Stuss and Knight, 2002) and in particular working memory (du Boisgueheneuc et al., 2006, Vestergaard et al., 2011). Functional MRI studies have identified abnormal functional connectivity of the left superior frontal gyrus and the anterior cingulate (Agam et al., 2010, Koshino et al., 2005, Koshino et al., 2008, Solomon et al., 2009, Solomon et al.,

2013). Disrupted white matter organisation of the left SLF I may contribute to this abnormal functional connectivity in ASD.

4.4.4 Disrupted Organisation and Lateralisation of the Superior Longitudinal Fasciculus II in ASD

Greater structural connectivity and rightward asymmetry of FA in the SLF II was observed in the ASD group relative to the control group. The SLF II structurally connects the anterior parietal sulcus and the angular gyrus to the posterior superior and middle frontal gyri / dorsolateral prefrontal cortex (DLPFC) (Thiebaut de Schotten et al., 2011). Connectivity of the DLPFC to both the inferior parietal cortex and anterior cingulate is considered a crucial component of cognitive control (Stuss and Knight, 2002). Interestingly, several studies have reported aberrant functional connectivity during cognitive control (Agam et al., 2010, Solomon et al., 2009), response inhibition (Kana et al., 2007) and response monitoring tasks in ASD (Thakkar et al., 2008). It is possible that disrupted organisation of white matter in the SLF may contribute to this functional dysconnectivity, however the links between structural and functional connectivity have not been well studied, are not well understood and have only been explicitly investigated in one previous study (McGrath et al., 2013b).

Research indicates that face processing is asymmetrically dominant in the right hemisphere (Kanwisher et al., 1997) and asymmetric myelination of white matter tracts has been shown to influence processing speed (Anderson et al., 1999). Right frontal regions connected via the SLF II are thought to play a role in non-verbal communication such as facial recognition (de Schonen et al., 2005) and processing of facial expressions (Ahs et al., 2014). Difficulty distinguishing facial expressions is a

common impairment reported in ASD (Lozier et al., 2014). It is possible that greater rightward asymmetry and complexity of the SLF II may result in a loss of integrated neural signalling when processing faces. During exploratory correlation analyses, the current study found an association between greater rightward asymmetry of FA in the SLF II and greater social and communication deficits. The SLF II connects brain regions involved in a number of cognitive functions thus it is plausible that the disrupted organisation of white matter in the SLF II may contribute to the functional and behavioural deficits reported in previous studies.

4.4.5 Significance of Increased Structural Connectivity of the Superior Longitudinal Fasciculus Branches

In neurotypical individuals, increased white matter development and higher FA values with age are thought to reflect the onset of higher cognitive functioning in adolescence. Increased FA values in the SLF have been associated with improved cognitive performance in tasks of spatial working memory, attention orienting, set-shifting and cognitive control (Burzynska et al., 2011, Urger et al., 2014, Vestergaard et al., 2011). Furthermore, clinical studies have shown that increased FA is associated with improved spatial working memory (Karlsgodt et al., 2008) and episodic memory (Levitt et al., 2012) in schizophrenia. Therefore, it is possible that greater FA values in ASD may also represent enhanced cognitive functioning relative to controls.

Given the hypothesised association of the SLF branches with dorsal and ventral attention networks (DAN and VAN) (Thiebaut de Schotten et al., 2011), it could be postulated that the increased FA and CL values observed in the left SLF I and the right SLF II may contribute to the greater functional connectivity observed in the ASD group

relative to controls during an attention orienting task (Chapter 3). In the VAN, increased functional connectivity was observed between projections from the right parietal regions (right precuneus and the right temporoparietal junction) and the right frontal regions (right frontal, middle and inferior gyri, right superior medial and orbital gyri and the right anterior cingulate). Larger FA and CL values yielded in the right SLF II in addition to a greater rightward asymmetry of FA in the SLF II in the ASD group relative to controls suggests that composite white matter neural architecture may facilitate enhanced attention orienting in ASD in order to maintain neurotypical behaviour. Although there was significant overlap in participants, the study population for these two studies differed, therefore no direct correlations can be made. Reduced functional connectivity was observed the DAN, but this reduction was demonstrated between parietal-temporal regions such as the left ventral intraparietal sulcus and the left middle temporal gyrus rather than fronto-parietal regions connected by the SLF I. It is possible that the increased complexity of white matter microstructural organisation of the SLF branches may aid cognitive tasks such as attention orienting.

4.4.6 Contribution of Tract Segmentation Analyses

It has been suggested that some white matter abnormalities may be overlooked when averaging diffusivity metrics along a tract (Colby et al., 2012). Segmentation of white matter tracts can elucidate whether there are specific regions of abnormal FA that are driving whole-tract differences. Exploratory analyses in the current study established that extensive differences in FA in the left SLF I and the right SLF II are largely driven by axial or parallel diffusivity differences in ASD relative to controls. Reduced AD has been associated with axonal degeneration (Song et al., 2002)

therefore, increased AD in the SLF branches may represent the maintenance of or more organised axonal structure in these regions. Reduced radial diffusivity was identified in segments of both the left SLF I and the right SLF II. Reduced RD is thought to reflect greater myelination of fiber pathways (Song et al., 2002) therefore, sections of the SLF II may have greater myelination in ASD, which subsequently enhances the communication facilitated by these white matter bundles. These findings illustrate the complexity of disruption of white matter tracts and demonstrate the importance of the segmentation approach described above. If whole tract statistics are used, between-group differences in region specific parts of the SLF would have been missed. Collectively, these along tract statistics demonstrate that the increased FA present in the left SLF I and right SLF III is largely driven by increased axial diffusivity and to a lesser extent reduced radial diffusivity. These findings suggest that both aberrant axonal density and myelination influence abnormal structural connectivity in ASD.

4.4.7 Limitations

There were a number of limitations to this study. As in the previous study of functional connectivity during attention orienting, participants with ASD were limited to male, right-handed individuals with average/above-average IQ. Therefore, results are specific to this group and do not reflect all individuals on the spectrum. Only the branches of the SLF were isolated in this study. It is likely that other white matter tracts are disrupted in ASD thus additional tracts warrant investigation using CSD tractography methods. Although a subset of participants in the current study performed the attention orienting task (Chapter 3, Section 3.3.2), it is difficult to draw inferences from the findings due to the slightly different population. The tract

segmentation findings were reported as uncorrected for multiple comparisons thus only tentative conclusions can be drawn. Although the population in this study is relative large, greater sample size in addition to an endophenotypic approach which, differentiates behavioural features based on genotype, would help address this issue.

4.4.8 Conclusion

For the first time in ASD research, this study has used CSD based tractography to isolate the discrete branches of the SLF I, II and III bilaterally in ASD. This work has demonstrated that white matter microstructural organisation is disrupted in the left SLF I and the right SLF II in an ASD population. The abnormal structural connectivity in these tracts was characterised by increased FA in the right SLF II and increased CL in the left SLF I and right SLF II. Atypical functional connectivity has been described between fronto-parietal regions and it is possible that irregular white matter architecture may underpin neural network abnormalities. Greater FA values in the SLF have been linked with greater spatial working memory, attention orienting, set-shifting and cognitive control (Burzynska et al., 2011, Urger et al., 2014, Vestergaard et al., 2011). The branches of the SLF are thought to link the key nodes of the dorsal and ventral attention networks, therefore increased functional connectivity previously reported during attention orienting (Chapter 3, Section 3.4.3.2) may be aided by an increased level of white matter organisation in the SLF I and the SLF II. Segmentation analyses revealed that increased FA was driven largely by an increase in AD and reduced RD to a lesser extent. Understanding the pathophysiology of ASD in terms of white matter development has crucial implications in terms of evaluating therapeutic interventions and establishing biomarkers for the disorder.

5 Whole Brain Analysis of White Matter Structure in ASD

5.1 Introduction

5.1.1 Typical White Matter Development

Diffusion MRI studies have established that the organisation of white matter microstructure, commonly referred to as structural connectivity, changes across the lifespan (Lebel et al., 2008, Asato et al., 2010, Bava et al., 2010, Qiu et al., 2008, Giorgio et al., 2008, Giorgio et al., 2010, Lebel and Beaulieu, 2011, Clayden et al., 2012, Wang et al., 2012a). These studies have consistently demonstrated that FA increases during childhood into adolescence and plateaus in adulthood. It appears that the trajectory of white matter development is tract-specific whereby white matter tracts reached peak FA values at different ages (Lebel et al., 2008, Lebel and Beaulieu, 2011, Clayden et al., 2012). White matter microstructural development has been shown to differ between genders (Asato et al., 2010, Clayden et al., 2012, Lebel and Beaulieu, 2011, Wang et al., 2012a) and is also related to intelligence (Wang et al., 2012a, Clayden et al., 2012). It is therefore important to consider gender, age and IQ when interpreting results from these studies.

5.1.2 Link between Functional and Structural Connectivity

It has been suggested that well-defined major white matter tracts underpin functional neural networks and cognitive processes. For example, language processing is supported by the arcuate fasciculus, which connects the traditional language centers

of Broca's area and Wernicke's area (Catani et al., 2005). The limbic network is involved in emotional processing and appears to be linked by a number of major white matter tracts including the cingulum, inferior longitudinal fasciculus and the arcuate fasciculus (Catani et al., 2002, Catani and Thiebaut de Schotten, 2008). The branches of the superior longitudinal fasciculus are thought to structurally connect brain regions within the dorsal and ventral attention networks (Thiebaut de Schotten et al., 2011) while the inferior fronto-occipital and arcuate fasciculi appear to connect neural regions involved in visuospatial processing (Catani and Thiebaut de Schotten, 2008). The theory of abnormal cortical connectivity suggests that both structural and functional connectivity abnormalities contribute to the clinical deficits characteristic of ASD [see (Vissers et al., 2012) for review]. Disrupted functional connectivity during language comprehension (Kana et al., 2006), emotion recognition (Alaerts et al., 2013), attention orienting (Fitzgerald et al., 2014) and visuospatial processing (McGrath et al., 2012) have been illustrated in a number of ASD studies. A direct association between disrupted structural connectivity and abnormal functional connectivity has been demonstrated (McGrath et al., 2013b) thus it is plausible that some of the atypical cognitive functions in ASD may be underpinned by disrupted organisation of white matter microstructure.

5.1.3 About Tract Based Spatial Statistics

Tract-based spatial statistics (TBSS; http://www.fmrib.ox.ac.uk/fsl/tbss/) is a method that was developed to overcome issues with registration, smoothing and partial volume effects, which may have marred results of previous voxel-based white matter studies (Smith et al., 2006). TBSS performs automated data-driven analysis of

white matter organisation using non-linear registration and projection of individual subject FA data onto a common FA skeleton thus eliminating any variation within voxels at the extremities of the white matter. Four diffusion metrics are typically evaluated using TBSS, fractional anisotropy (FA), axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD) (see Sections 1.5.3.2 and 2.4.3). TBSS has been used to evaluate white matter architecture in a number of neuropsychiatric disorders including schizophrenia (Ellison-Wright et al., 2014, Holleran et al., 2014), 22q11.2 syndrome (Kikinis et al., 2012), attention deficit/hyperactivity disorder (Wang et al., 2012b) and depression (Zuo et al., 2012, Aghajani et al., 2013). These studies have illustrated the sensitivity of this analytic approach for detecting white matter abnormalities in clinical populations.

5.1.4 Tract Based Spatial Statistics Studies of ASD

Since the development of this method, a number of TBSS studies have been performed in ASD across different stages of the lifespan. In early childhood (aged < 6 years), increased FA was detected in the corpus callosum (Weinstein et al., 2011a, Billeci et al., 2012), superior longitudinal fasciculus (Weinstein et al., 2011a), arcuate fasciculus (Billeci et al., 2012), the cingulum (Weinstein et al., 2011a, Billeci et al., 2012) and the internal and external capsules (Billeci et al., 2012). On the other hand, in late childhood (aged between 6 and 12 years), reduced FA was observed in a number of tracts including the anterior thalamic radiation (Cheon et al., 2011, Barnea-Goraly et al., 2010), corpus callosum (Cheon et al., 2011, Jou et al., 2011b, Barnea-Goraly et al., 2010), uncinate fasciculus (Cheon et al., 2011, Jou et al., 2011b), cingulum (Jou et al., 2011b, Barnea-Goraly et al., 2010), superior longitudinal fasciculus (Barnea-Goraly et

al., 2010) inferior fronto-occipital fasciculus (Jou et al., 2011b), inferior longitudinal fasciculus (Jou et al., 2011b, Cheon et al., 2011), frontal corona radiata (Barnea-Goraly et al., 2010) forceps major (Jou et al., 2011b), forceps minor (Jou et al., 2011b), internal capsule (Barnea-Goraly et al., 2010) and corticospinal tract (Jou et al., 2011b) while another study demonstrated no difference in FA at this stage of development (Ameis et al., 2011). Increased MD (Ameis et al., 2011, Cheon et al., 2011), increased RD (Ameis et al., 2011) and reduced AD (Barnea-Goraly et al., 2010) were also recorded in some of these reports of children aged between 6 and 12 years.

In adolescents (aged between 12 and 18 years), results were similar to the 6 -12 age range cohort in that reduced FA was found in the corpus callosum (Shukla et al., 2011, Bakhtiari et al., 2012), internal capsule (Cheng et al., 2010, Shukla et al., 2011), inferior longitudinal fasciculus (Shukla et al., 2011, Bakhtiari et al., 2012) superior longitudinal fasciculus (Cheng et al., 2010, Shukla et al., 2011, Bakhtiari et al., 2012), inferior fronto-occipital fasciculus (Shukla et al., 2011, Bakhtiari et al., 2012), uncinate fasciculus (Bakhtiari et al., 2012), cingulum (Shukla et al., 2011, Bakhtiari et al., 2012), anterior thalamic radiation (Shukla et al., 2011, Bakhtiari et al., 2012), corticospinal tract (Shukla et al., 2011, Bakhtiari et al., 2012), forceps major and minor (Bakhtiari et al., 2012) and inferior cerebellar peduncle (Cheng et al., 2010) while increased FA was identified in the superior corona radiata, anterior thalamic radiation, inferior occipito-frontal fasciculus, middle cerebellar peduncle and different regions of the internal capsule and superior longitudinal fasciculus (Cheng et al., 2010) as well as the optic radiation and superior fronto-occipital fasciculus (Bode et al., 2011).

In adults (aged > 18), widespread reduced FA was identified in the corpus callosum, tapetum, uncinate fasciculus, superior fronto-occipital fasciculus, superior

longitudinal fasciculus, internal capsule, posterior thalamic radiation, posterior and superior corona radiata and the fornix (Gibbard et al., 2013, Kleinhans et al., 2012) in addition to the cingulum, cerebellar peduncle, anterior corona radiata and corticospinal tract (Kleinhans et al., 2012) although one study found no between group difference (Bakhtiari et al., 2012).

The reduced FA reported in the majority of studies, regardless of age appeared to be characterised by increased MD (Shukla et al., 2011, Gibbard et al., 2013, Kleinhans et al., 2012), increased RD (Shukla et al., 2011, Cheng et al., 2010, Gibbard et al., 2013, Kleinhans et al., 2012) and reduced AD (Cheng et al., 2010) while in studies with increased FA, reduced RD (Weinstein et al., 2011a, Cheng et al., 2010) and increased AD were observed (Cheng et al., 2010). Overall, the results of TBSS studies to date suggest that FA may be greater in early childhood followed by a reduction in FA with age in ASD relative to controls however a pattern of white matter abnormality has not yet been determined.

5.1.5 Tract Based Spatial Statistics and Correlations with Behaviour

To investigate whether abnormal white matter development in ASD has clinical implications, correlations with behavioural measures of ASD characteristics were performed in some of these studies. Greater communication deficits measured by the ADOS and ADI subscales were associated with reduced FA in the inferior fronto-occipital and superior longitudinal fasciculi while higher social impairment was correlated with lower FA values in the inferior longitudinal fasciculus (Bakhtiari et al., 2012). Gibbard et al. (2013) illustrated an association with diffusion metrics and autism

quotient (AQ) scores. FA values were negatively correlated with higher AQ scores while MD and RD were positively correlated. Another study demonstrated that reduced FA in the anterior thalamic radiation and uncinate fasciculus was associated with higher total scores on the social responsiveness scale (Cheon et al., 2011) while some studies demonstrated that FA values were not associated with autism symptomology (Kleinhans et al., 2012, Barnea-Goraly et al., 2010, Jou et al., 2011b). Given the problems that arise from using ADI and ADOS scores for correlation analyses (see Chapter 2, Section 2.6.5), the relationship between FA and clinical symptomology is unclear. By using biologically valid measures of social and communication deficits and restricted repetitive behaviours (see Chapter 2, Section 2.6.5 and Appendix B for details), this study sought to clarify the association between white matter abnormalities and clinical traits of ASD.

5.1.6 Limitations of Previous Tract Based Spatial Statistics Studies of ASD

Although the TBSS analytic method is relatively homogeneous, studies have shown reduced FA values (Cheon et al., 2011, Jou et al., 2011b, Barnea-Goraly et al., 2010, Bakhtiari et al., 2012, Cheng et al., 2010, Gibbard et al., 2013, Kleinhans et al., 2008, Shukla et al., 2011), greater FA values (Weinstein et al., 2011a, Billeci et al., 2012, Bode et al., 2011, Cheng et al., 2010) and no difference in FA (Ameis et al., 2011, Bakhtiari et al., 2012) thus results do not provide a clear pattern of white matter abnormality in ASD. It is possible that the discrepancies reflect the use of different DTI acquisition protocols and preprocessing methodologies (Keedwell et al., 2012). In DTI acquisition protocols, FA values are affected by the number of diffusion-weighted

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 thus a minimum N of > 20 is recommended (Giannelli et al., 2010). Notably, a number of previous TBSS studies in ASD have used fewer than 20 diffusion weighted directions (Ameis et al., 2011, Cheng et al., 2010, Weinstein et al., 2011b, Shukla et al., 2011, Barnea-Goraly et al., 2010).

Typical preprocessing of diffusion data involves correcting for eddy current induced distortions and 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 previous studies using TBSS in ASD mention B matrix reorientation in their methods.

Cardiac pulsation commonly leads to artifact in clinical DTI acquisitions. RESTORE (Robust Estimation of Tensors by Outlier Rejection) is a preprocessing algorithm for DTI data that offers an effective approach to obtain diffusion tensor parameters immune from cardiac induced artifacts (Chang et al., 2005). Artifacts due to cardiac pulsation were also not mentioned in previous TBSS analyses in ASD.

It is also important to note that a number of the studies cited above contained small samples (< 18) (Barnea-Goraly et al., 2010, Cheon et al., 2011, Jou et al., 2011b),

both genders (Kleinhans et al., 2012, Gibbard et al., 2013, Bakhtiari et al., 2012, Shukla et al., 2011, Barnea-Goraly et al., 2010, Ameis et al., 2011) and were not matched on IQ (Billeci et al., 2012, Barnea-Goraly et al., 2010), factors which are likely to have influenced results.

The current study attempted to address these issues by obtaining diffusion-weighted data with 61 directions, implementing data preprocessing steps which incorporated the B-matrix and RESTORE algorithm in ExploreDTI and recruiting a large sample (the largest in a TBSS study of ASD to date) of male participants who were matched for both age and IQ.

5.1.7 Aims

The main objectives of this study were to:

1. Use the TBSS methodology to investigate white matter microstructural organisation in a well-defined sample of individuals with ASD and age and IQ-matched neurotypical controls.
2. Evaluate age-related changes in diffusion measures of FA, AD, RD and MD between the ASD and control groups.
3. Determine if there is an association between clinical deficits in ASD and white matter microstructural differences between groups.

5.2 Methods

5.2.1 Participants

Participants included in this analysis were taken from the 'McGrath' and 'Fitzgerald' samples. Details of the participants for this study (study 3) are described in Table 5.1 and in Chapter 2, Section 2.2. Distribution of social and communication deficits and restricted repetitive behaviours are the same as those presented in Chapter 4, Figures 4.1 and 4.2. Tests of normal distribution are also reported in Chapter 4, Table 4.2.

Table 5.1. Participant Demographics

	Control	**ASD**	**P-value**
Number	45	45	
Gender	Male	Male	
Mean age ± SD; range	16.55 ± 3.04; 10.25-24.2	15.91 ± 3.3; 10-24	0.336
Mean Full Scale IQ ± SD; range	115 ± 14.62; 81-147	109.5 ± 15.9; 78-145	0.098
Social Communication Deficits		24.29 ± 6.82: 12-40	
Restricted Repetitive Behaviours		4.87 ± 2.53: 0-12	
ADOS: Social and Communication		9.82 ± 2.871; 7-19	

5.2.2 Diffusion-Weighted Imaging Preprocessing

Preprocessing of diffusion data was completed using ExploreDTI software (http://www.ExploreDTI.com) (Leemans et al., 2009). Details of the preprocessing

pipeline are described in Chapter 4, Section 4.3.2. Briefly, each dataset was corrected for eddy current and echo planar imaging distortions as well as subject motion. The B-matrix rotation was included in the preprocessing pipeline to maintain orientation of the data (Leemans and Jones, 2009). The tensor model was applied using the RESTORE approach to minimise artefacts (Chang et al., 2005). FA, AD, RD and MD were extracted from the preprocessed data for every subject.

5.2.3 Tract-based Spatial Statistics

Whole brain voxel-wise analysis of microstructural organisation of white matter was performed using Tract-based Spatial Statistics (TBSS) (Smith et al., 2006) within FSL FMRIB Software (Smith et al., 2004) version 4.1.9. The FA diffusion images were extracted from the preprocessing pipeline in ExploreDTI. The images were additionally preprocessed in FSL to remove outliers from the diffusion tensor fitting. A study specific template incorporating all subjects was created by aligning every FA image per subject to every other FA image per subject to identify the most representative image. 1x1x1 mm resolution is optimum for subsequent skeletonisation and projection steps; therefore the study specific template is then affine-aligned into 1x1x1 mm MNI 152 standard space. Every other subject image was transformed into 1x1x1 mm MNI standard space by combining the non-linear transformation to the target FA image with the affine transformation from the template to MNI 152 space in a single step to avoid re-sampling the image twice. A mean FA skeleton was then derived by projecting the thinned FA images for each subject onto a single skeleton image. The mean FA skeleton represents all white matter tracts across groups. The resulting data was then used for subsequent voxel-wise cross-subject statistics. TBSS was also used to

investigate MD, AD and RD by applying the non-linear warps and skeleton projection generated during the FA TBSS pipeline to the AD, RD and MD diffusion images that were also extracted from ExploreDTI.

5.2.4 Statistical Analysis

Permutation based methods were used to investigate between group differences in FA, AD, RD and MD. Permutation approaches are used for inference or thresholding when the null distribution is unknown and are effective in controlling against false positives (Winkler et al., 2014). “Randomise” is a permutation program in FSL which thresholds statistical images using a standard general linear model design. Independent t-tests were run using randomise to examine between group differences in the four diffusion metrics. Age and intracranial volume were demeaned and included in the model. Statistical thresholding was applied using threshold-free cluster enhancement (TFCE) with 10000 permutations at $p < 0.05$, which is sensitive to spatially extensive areas of significance difference (Smith and Nichols, 2009). The anatomic location of each cluster that showed a significant between-group difference in the diffusion metrics was determined using the atlas tool in FSLView (http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions), which incorporates a number of anatomic templates including the Harvard-Oxford Cortical and Subcortical Structural Atlases, Johns Hopkins University DTI-based white matter atlases, Talairach atlas, MNI structural atlas and Oxford thalamic connectivity atlas. Mean values of FA, AD, RD and MD were extracted from regions of statistical significance for correlation analyses.

5.2.5 Correlation Analyses

Bivariate Spearman correlations were performed to investigate the relationship between clinical characteristics of ASD, behavioural performance of executive functioning tasks and white matter microstructural differences in ASD. For diffusion measures showing significant between group differences, correlation analyses were carried out investigating relationships between these measures and social and communication deficits and restricted repetitive behaviours. A two-factor model was derived using items from the Autism Diagnostic Interview-Revised to evaluate social, communication and restricted and repetitive behavioural impairments (Georgiades et al., 2013) (See Chapter 2, Section 2.6.5).

A subset of the participants performed an attention-orienting task (Chapter 3, Section 3.3.2). Behavioural performance was measured in terms of the 'Validity Effect' (Invalid – Valid mean response time) for the attention task. Pearson correlation analyses were carried out in this subset to determine if behavioural performance was associated with aberrant white matter organisation (n = 21 ASD, 21 controls). Bonferroni correction of correlation analyses were performed based on the number of measures used (p =0.05/3 = 0.016).

5.3 Results

5.3.1 Diffusion Metrics

Reduced fractional anisotropy was observed in the ASD group in the bilateral genu, body and splenium of the corpus callosum, the bilateral anterior and posterior corona radiata and the bilateral anterior thalamic radiation in contrast to the control group (Table 5.2 and Figure 5.1). The ASD group also showed reduced fractional anisotropy in the right superior longitudinal fasciculus, the forceps minor and major as well as the left cingulum, inferior longitudinal fasciculus and the inferior fronto-orbital fasciculus relative to controls. The ASD group did not have greater fractional anisotropy in any white matter region than the controls. No significant between group differences were observed in radial, axial or mean diffusivity.

Table 5.2. Between-Group Differences in Diffusion Metrics

	White Matter Region	X	Y	Z	ASD ± SD	Control ± SD	P-value
	Fractional Anisotropy						
Right	Genu of the Corpus Callosum	15	22	24	0.545 ± 0.067	0.587 ± 0.046	0.001
	Body of the Corpus Callosum	15	12	31	0.481 ± 0.076	0.516 ± 0.065	0.02
	Splenium of the Corpus Callosum	20	-43	26	0.531 ± 0.06	0.561 ± 0.047	0.011
	Anterior Corona Radiata	17	22	28	0.424 ± 0.047	0.453 ± 0.046	0.003
	Posterior Corona Radiata	29	-54	23	0.418 ± 0.068	0.474 ± 0.085	0.001
	Anterior Thalamic Radiation	11	-18	16	0.349 ± 0.039	0.378 ± 0.031	0.000
	Superior Longitudinal Fasciculus	33	-27	34	0.525 ± 0.076	0.568 ± 0.069	0.006
	Forceps Minor	16	37	2	0.611 ± 0.056	0.636 ± 0.048	0.024
	Forceps Major	28	-62	15	0.703 ± 0.089	0.737 ± 0.068	0.043
Left	Genu of the Corpus Callosum	-14	27	21	0.543 ± 0.069	0.572 ± 0.065	0.042
	Body of the Corpus Callosum	-15	13	28	0.549 ± 0.078	0.595 ± 0.063	0.003
	Splenium of the Corpus Callosum	-18	-45	26	0.485 ± 0.059	0.514 ± 0.06	0.022
	Anterior Corona Radiata	-17	38	14	0.423 ± 0.068	0.451 ± 0.062	0.042
	Superior Corona Radiata	-25	-30	24	0.53 ± 0.048	0.555 ± 0.059	0.028
	Anterior Thalamic Radiation	-3	-11	13	0.335 ± 0.046	0.367 ± 0.04	0.042
	Cingulum	-8	-3	35	0.668 ± 0.071	0.7 ± 0.066	0.03
	Inferior Longitudinal Fasciculus	-32	-57	20	0.494 ± 0.068	0.523 ± 0.068	0.045

All reported regions are corrected for multiple comparisons, $p < 0.05$. X, Y, Z = Peak Montreal Neurological Institute (MNI) co-ordinates, SD = Standard Deviation

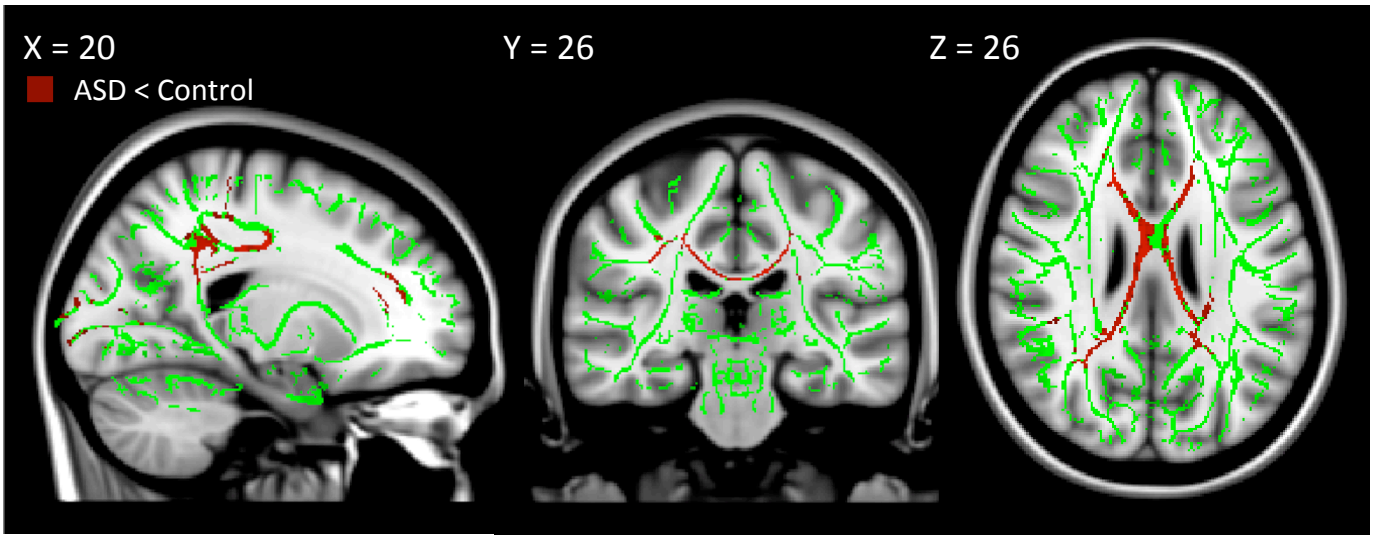

Figure 5.1. Voxelwise Between-Group Differences in Fractional Anisotropy

Reduced FA in the ASD group is shown in red. Images are displayed in radiological convention (left is right). All data is corrected for multiple comparisons, $p < 0.05$.

5.3.2 Group-by-Age Interaction with Fractional Anisotropy

There was a significant group-by-age interaction in the left anterior thalamic radiation (MNI: -3 -11 13; p = 0.02) (Figure 5.2). The ASD group showed a significant increase in fractional anisotropy with age (r = 0.361, p = 0.015) whereas the control group did not show any significant change (r = 0.026, p = 0.868).

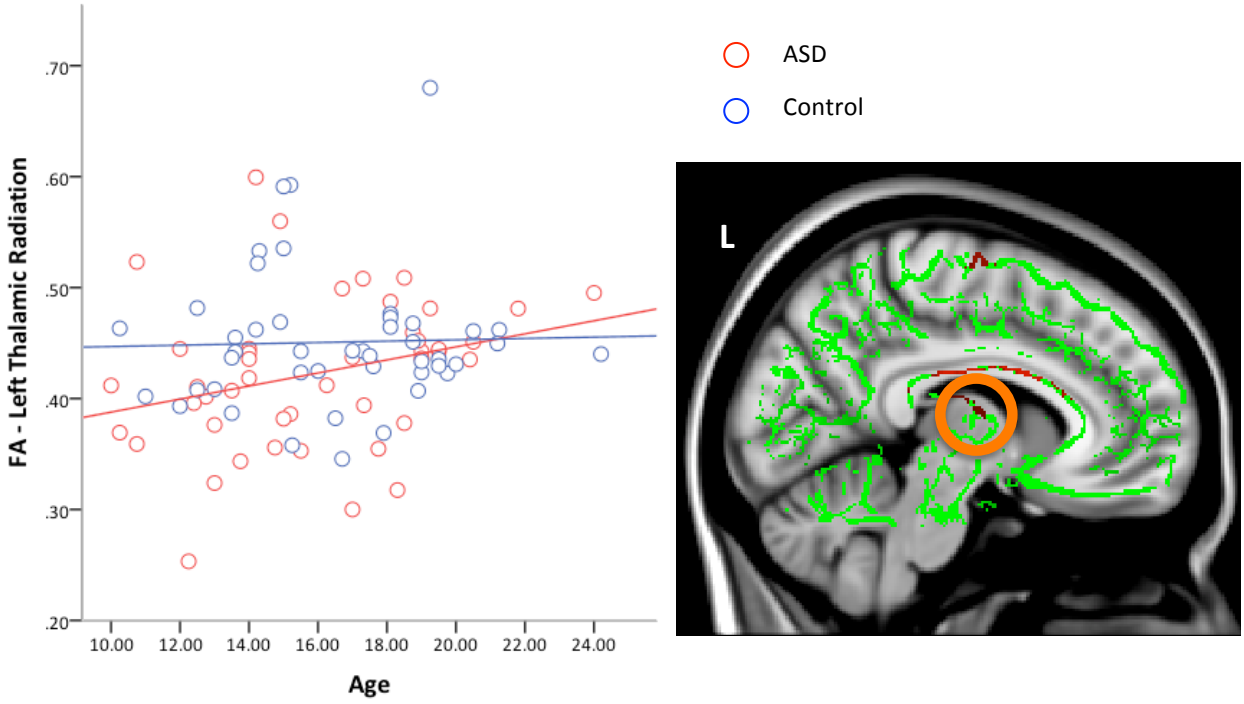

Figure 5.2. Group-by-age Interaction Effect of Fractional Anisotropy in the Left Anterior Thalamic Radiation

The region of the left anterior thalamic radiation showing a group-by-age interaction effect of FA is circled in orange, corrected for multiple comparisons, p < 0.05, L = Left

5.3.3 Correlation Analyses

In ASD, reduced FA in the bilateral body of corpus callosum (right; r = -0.413, p = 0.005, left; r = -0.485, p = 0.001), right anterior thalamic radiation (r = -0.323, p = 0.031) and the left anterior corona radiata (r = -0.329, p = 0.027) was associated with greater deficits in social communication according to the score derived from the two-factor ADI model. Greater restricted repetitive behaviours was correlated with lower FA in the right body of the corpus callosum (r = -0.366, p = 0.013) and the right superior longitudinal fasciculus (r = -0.367, p = 0.013). Behavioural performance on the attention orienting task, i.e. the validity effect, was correlated with the left superior corona radiata (r = -0.427, p = 0.037) and the left anterior corona radiata (r = -0.447, p = 0.028) in the ASD group. Reduced FA in these regions was associated with poorer behavioural performance during the task. There was no correlation between validity effect and white matter differences in the control group.

Table 5.3. Correlation Analyses of Behavioural Measures and Between-Group White Matter Differences of Fractional Anisotropy

	White Matter Region	SCD (r; p value)	RRB (r; p value)	Validity effect (r; p value)
	Fractional Anisotropy			
Right	Body of the Corpus Callosum	**-0.413****; 0.005	**-0.366****; 0.013	0.186; 0.385
	Anterior Thalamic Radiation	-0.323*; 0.031	-0.141; 0.354	0.257; 0.225
	Superior Longitudinal Fasciculus	-0.076; 0.617	**-0.367****; 0.013	0.145; 0.498
Left	Body of the Corpus Callosum	**-0.485****; 0.001	-0.223; 0.142	0.093; 0.664
	Anterior Corona Radiata	-0.329*; 0.027	-0.268; 0.076	0.447*; 0.028
	Superior Corona Radiata	-0.084; 0.582	-0.043; 0.781	0.427*; 0.037

** Bonferroni corrected for number of measures, $p < 0.05/3 = 0.016$, * Uncorrected for multiple clinical measures, SCD = Social and communication deficits, RRB = Restricted repetitive behaviours, Validity Effect = Performance during attention orienting task

5.4 Discussion

5.4.1 Overview of Findings

This whole brain study has demonstrated that white matter microstructural organisation is atypical in a large well characterised cohort of individuals with ASD relative to carefully matched neurotypical controls. Widespread bilateral white matter anomalies were observed in the genu, splenium and body of the corpus callosum, anterior thalamic radiation, posterior and anterior corona radiata, right superior longitudinal fasciculus, right forceps major and minor, left cingulum and the left inferior longitudinal fasciculus. Additionally, age-related atypical white matter development was demonstrated in the left anterior thalamic radiation. Furthermore, disrupted white matter organisation was associated with behavioural impairments frequently observed in ASD. These results replicate the findings from a number of previous studies investigating white matter organisation in adolescents in ASD (Shukla et al., 2011, Bakhtiari et al., 2012, Cheng et al., 2010). Thus it is plausible that the brain regions identified in the current study reflect genuine areas of white matter pathology in ASD.

5.4.2 Considerations of the Tract-based Spatial Statistics Method

In the present study, optimal data acquisition parameters and preprocessing steps incorporating the B-matrix and RESTORE algorithm in ExploreDTI were used to provide the highest quality data for statistical analysis. However, recent methodological research has revealed a number of limitations of the standard TBSS

analytic approach. Bach et al. (2014) reported general methodological considerations for TBSS, which are not currently incorporated into the standard processing pipeline used in the current study. This paper indicated that the standard approach is susceptible to anatomical inaccuracies and bias during the skeleton projection step and noise-dependent FA skeleton irregularities, which may affect the reliability of TBSS results. Other research illustrated that the statistical sensitivity of the FA skeleton is not optimal (Edden and Jones, 2011) and may be underpowered to detect significant between-group differences with small samples and/or subtle effects (Cykowski et al., 2011).

It has also been well documented that the diffusion tensor model cannot describe the diffusion correctly in regions of complex fiber architecture (voxels which contain fibers of multiple orientations) (see Chapter 4, Section 4.1.3 for more details). Due to the high prevalence of voxels containing crossing fibers in the brain, interpretation of white matter abnormalities using diffusion tensor-derived metrics can be problematic.

Despite these limitations, TBSS has been used as a valid approach to investigate white matter abnormalities in ASD. Several studies of ASD have used this method and identified disrupted white matter organisation of a number of fiber pathways. Although a number of studies have performed TBSS analyses in ASD, no clear pattern of pathology has emerged. This is most likely due to varying data acquisition parameters, inadequate preprocessing protocols, lack of statistical power as well as the inclusion of different age groups, both genders and unmatched cohorts. The current study followed best practice parameters and identified a number of white matter abnormalities. The following section discusses these findings in the context of

previous literature and also outlines the possible functional effects associated with these white matter anomalies.

5.4.3 Corpus Callosum

The corpus callosum (CC) is a major interhemispheric white matter tract that structurally connects both hemispheres. The genu of the CC links frontal regions bilaterally, the body of the CC structurally connects bilateral parietal regions and the splenium of the CC provides interhemispheric connections between posterior parietal and occipital regions. In this study, reduced FA was demonstrated bilaterally in the genu, body and splenium of the corpus callosum. The findings of the current study are in keeping with a number of other studies investigating white matter in ASD. Previously, reduced FA was observed in the CC in voxel-based whole brain studies (Bloemen et al., 2010, Jou et al., 2011a, Noriuchi et al., 2010), TBSS studies (Shukla et al., 2011, Bakhtiari et al., 2012, Kleinhans et al., 2012, Barnea-Goraly et al., 2010, Cheon et al., 2011, Gibbard et al., 2013, Jou et al., 2011b) and in DTI tractography studies which specifically isolated the CC (Kumar et al., 2010, Lo et al., 2011). Furthermore, meta-analyses investigating the structure of the CC in autism found a reduction in CC volume (Radua et al., 2011), area and size (Frazier and Hardan, 2009, Stanfield et al., 2008) in which the effect was greater in anterior regions. MRI functional connectivity studies also illustrate that neural regions functionally connected via the CC are aberrant in ASD (Just et al., 2007, Kana et al., 2006, Mason et al., 2008). Reduced CC size has been associated with reduced functional connectivity during executive functioning (Just et al., 2007), theory of mind (Mason et al., 2008), sentence comprehension (Kana et al., 2006) and at rest (Cherkassky et al., 2006).

Clinical symptomology of ASD has been linked with corpus callosum structural abnormality. Greater social and communication deficits have been associated with reduced CC volume (Prigge et al., 2013), reduced fiber density in the CC (Kumar et al., 2010) and agenesis of the CC (Lau et al., 2013). The current study demonstrated that greater social and communication deficits and restricted repetitive behaviours were associated with reduced FA in the body of the CC. This finding supports the theory that disrupted organisation of white matter in the CC may impact on the development of core deficits in ASD.

5.4.4 Anterior Thalamic Radiation

The anterior thalamic radiation (ATR) is a major white matter tract, which projects through the anterior limb of the internal capsule and connects the thalamus, prefrontal cortex and striatum, which participate in prefrontal-subcortical circuits (Behrens et al., 2003). The ATR is likely to play an important role in central information processing (Herrero et al., 2002), executive functioning (Tekin and Cummings, 2002) and awareness (Van der Werf et al., 2002). In this study, reduced FA in the ATR was reported. Disrupted white matter organisation in the ATR has been reported in a number of other TBSS studies of ASD (Bakhtiari et al., 2012, Barnea-Goraly et al., 2010, Cheon et al., 2011, Shukla et al., 2011). Abnormal thalamo-cortical functional connectivity has been reported in ASD (Kleinhans et al., 2008, Mostofsky et al., 2009, Mizuno et al., 2006, Nair et al., 2013, Shih et al., 2011) and research has indicated that ASD symptoms are associated with both abnormal functional and structural connectivity of the thalamic circuits. For example, greater social interaction deficits were associated with reduced FA in the anterior thalamic radiation (Cheung et al.,

2009), abnormal fronto-thalamic structural connectivity (Nair et al., 2013) and aberrant temporo-thalamic functional connectivity (Nair et al., 2013). Correlation findings of a link between reduced FA and greater social and communication deficits align with those of another study (Cheung et al., 2009) thus highlighting the potential influence of aberrant white matter organisation of the anterior thalamic radiation in ASD symptomology.

An age-related difference in white matter trajectory was found in the left ATR where FA increased with age in the ASD group but not in the control group. Other studies investigating age-related changes in white matter development found mixed results. Some studies demonstrated that FA both increased (Shukla et al., 2011, Cheng et al., 2010) and decreased (Kleinhans et al., 2012) with age in typically developing individuals but not in ASD. However, none of these studies found age-related differences in the anterior thalamic radiation between groups. These studies had fewer participants (Cheng et al., 2010, Kleinhans et al., 2012, Shukla et al., 2011), older adults (Kleinhans et al., 2012) and included females, both left and right handed individuals and medicated participants in their sample (Shukla et al., 2011). In neurotypically developing individuals, FA is considered to increase in late childhood/early adolescence and begin to plateau into adulthood but the rate and time span of white matter development is tract dependent (Giorgio et al., 2010, Lebel et al., 2008, Clayden et al., 2012, Asato et al., 2010, Bava et al., 2010). The finding from this study may reflect abnormal white matter growth in the ATR in individuals with ASD that continues into adulthood.

5.4.5 Corona Radiata

The anterior and posterior corona radiata consist of projection fibers which radiate out from the brain stem to the cerebral cortex via the anterior and posterior limb of the internal capsule respectively. The projection fiber system differentiates into two principal systems; ascending fibers from the thalamus to the cerebral cortex and descending fibers from the fronto-parietal cortex to the subcortical nuclei including the basal ganglia and corticospinal tract (Catani and Thiebaut de Schotten, 2008) and is thought to facilitate perceptual, motor and higher cognitive functions (Schmahmann et al., 2008). The current results of reduced FA in the anterior, posterior and superior corona radiata support previous evidence of aberrant white matter organisation in the corona radiata in ASD (Kleinhans et al., 2012, Barnea-Goraly et al., 2010, Gibbard et al., 2013). The finding of a correlation between reduced FA in the left superior and anterior corona radiata and poorer performance during the executive function of attention orienting supports the view that abnormalities in the structure of the corona radiata may contribute to executive dysfunction in ASD. Furthermore, the findings suggest that the left anterior corona radiata may play a role in social and communication impairments in the disorder.

5.4.6 Right Superior Longitudinal Fasciculus

The superior longitudinal fasciculus (SLF) is a long-range association white matter tract, which connects parietal, frontal and temporal regions (Kamali et al., 2014). The SLF is thought to play an important role in language processing (Glasser and Rilling, 2008, Bernal and Altman, 2010, Gold et al., 2007, Brauer et al., 2011, Ashtari et al., 2007), spatial working memory (Vestergaard et al., 2011, Burzynska et al., 2011),

attention orienting (Ge et al., 2013), set-shifting (Urger et al., 2014) and cognitive control (Noble et al., 2013, Chaddock-Heyman et al., 2013). In the current study, a posterior region of the SLF within the parietal lobule showed abnormal microstructural organisation characterised by reduced FA. This finding adds to literature that has previously reported abnormal white matter in this tract in ASD (Barnea-Goraly et al., 2010, Cheng et al., 2010, Shukla et al., 2011, Kumar et al., 2010, Bakhtiari et al., 2012, Gibbard et al., 2013, Kleinhans et al., 2012). Reduced FA in this region of the SLF was also associated with greater restricted repetitive behaviours suggesting that white matter anomalies in the SLF may facilitate greater rigidity in autism. In ASD, functional neuroimaging studies have demonstrated reduced functional connectivity during tasks interrogating mental imagery (Kana et al., 2006), executive function (Just et al., 2007), cognitive control (Solomon et al., 2009) and visuospatial processing (Damarla et al., 2010). Structural connectivity of the SLF has been suggested to underpin these processes (Noble et al., 2013, Chaddock-Heyman et al., 2013, Urger et al., 2014). Therefore it can be postulated that abnormal white matter architecture in the SLF may contribute to behavioural characteristics of ASD.

5.4.7 Right Forceps Major and Minor

The forceps major is a fiber bundle, which connects the occipital lobes and transects the midline via the splenium of the corpus callosum while the forceps minor connects the lateral and medial frontal regions across the midline via the genu of the corpus callosum. The forceps major appears to play an important role in visual perception, processing visuospatial information, memory and topographical orientation (Tamura et al., 2007) and disrupted functional connectivity during

visuospatial processing has been reported in ASD (McGrath et al., 2012). The forceps minor is involved in execution function (Miller and D'Esposito, 2005), and aberrant functional connectivity has been reported during executive function tasks in ASD (Just et al., 2007). The finding of reduced FA in both the forceps major and minor in this study is in keeping with the results of two prior studies which used the TBSS approach to study white matter structure in a similar population of male adolescents (Jou et al., 2011b, Bakhtiari et al., 2012). Other studies did not find differences in white matter organisation in these tracts (Thomas et al., 2011, Weinstein et al., 2011a, Wolff et al., 2012). These studies included young children and older adults suggesting that differences in the forceps major and minor may be related to age.

5.4.8 Left Cingulum

The results reported in the present study of reduced FA in the left cingulum replicate previous findings of white matter anomalies in TBSS studies (Jou et al., 2011b, Barnea-Goraly et al., 2010, Kleinhans et al., 2012) and tractography studies (Billeci et al., 2012, Ikuta et al., 2014) of the cingulum in ASD. The cingulum is a long medial association fiber within the cingulate gyrus that extends along its length from the orbitofrontal cortex into the parahippocampal gyrus and is defined dorsally by the corpus callosum projecting into the temporal lobe along the ventral/medial wall of the hippocampal gyri. The cingulum is part of the limbic system that is involved in attention, memory and emotional processing (Catani, 2006, Rudrauf et al., 2008) and disrupted functional connectivity during these cognitive processes has been illustrated in ASD [see (Vissers et al., 2012) for review]. A study by McGrath et al. (2013b) demonstrated that regions that are abnormally functional connectivity are directly

linked by disrupted white matter tracts. This suggests that that irregular white matter architecture, such as in within the cingulum may be linked to the functional deficits that are characteristic of ASD.

5.4.9 Left Inferior Longitudinal Fasciculus

The inferior longitudinal fasciculus (ILF) is a major ventral fiber tract that projects from the visual association cortex through the inferior temporal lobe to the anterior temporal pole. Short fibers of the ILF connect visual areas to subcortical structures, the hippocampus and the amygdala. The ILF is thought to facilitate language production by mediating visual information (Mandonnet et al., 2007), visual perception (Ffytche, 2008, ffytche and Catani, 2005), face recognition (Fox et al., 2008), face processing (Suzanne Scherf et al., 2013), visual memory (Ross, 2008) and object recognition (Ortibus et al., 2012). Consistent with several studies that have reported abnormalities of the ILF in ASD (Jou et al., 2011b, Cheon et al., 2011, Bakhtiari et al., 2012, Shukla et al., 2011), individuals with ASD in this study showed altered white matter structure in the right ILF characterised by reduced FA. However, other studies failed to find differences of FA in this tract, but described increased left lateralisation of tract volume (Thomas et al., 2011) and increased number of streamlines in ASD (Pugliese et al., 2009) demonstrating that white matter anomalies in the ILF exist in ASD yet need to be defined further.

5.4.10 Interpretation of Overall Findings

Although widespread reduced FA was observed in this study in ASD, differences in axial diffusivity (AD), radial diffusivity (RD) or mean diffusivity (MD) were not demonstrated. As FA is considered to be a non-specific indicator of white matter

microstructural organisation it is not possible to comment on the specific white matter pathology present. Lower FA values are broadly thought to represent demyelination, axonal damage or reduced white matter coherence (Alexander et al., 2007b). As mentioned in earlier chapters, reduced AD is believed to reflect aberrant axonal integrity, increased RD is considered indicative of demyelination (Song et al., 2002, Song et al., 2005) and increased MD is thought to represent demyelination and axonal loss (Alexander et al., 2007b). Previous studies in ASD have demonstrated increased MD (Billeci et al., 2012, Shukla et al., 2011, Kleinhans et al., 2012, Cheng et al., 2010, Ameis et al., 2011, Gibbard et al., 2013), increased RD (Shukla et al., 2011, Kleinhans et al., 2012, Cheng et al., 2010, Ameis et al., 2011, Gibbard et al., 2013) and reduced AD (Cheng et al., 2010, Noriuchi et al., 2010). It may be that aberrant AD, RD and MD contribute equally to the white matter microstructural abnormalities observed in the current study and none independently underpin the FA differences in ASD observed in this study.

Using optimal quality high angular resolution diffusion data and preprocessing methods, disrupted white matter microstructure was illustrated using the standardised TBSS analytic approach. Despite methodological drawbacks, it is clear that TBSS can be used to identify regions of aberrant structural connectivity in ASD. Abnormal structural connectivity was demonstrated in several prominent white matter tracts such as the corpus callosum, anterior thalamic radiation and the corona radiata which have been linked to functional deficits in ASD. However, further research is required to understand what is driving these structural differences. Newer methodological approaches such as CSD based tractography and tract segmentation analyses may help elucidate these structural differences in ASD.

5.4.11 Limitations

As mentioned in the previous chapters, only high functioning male participants were included in the analysis thus findings cannot be attributed to all individuals with ASD. The trajectory of white matter development changes throughout the lifespan and findings from the current study are limited to interpretation within an adolescent cohort. Limitations of the TBSS approach which have previously outlined (see Section 5.5.2) may also hinder interpretation of the findings.

5.4.12 Conclusion

A tract based spatial statistical analysis of high quality diffusion MRI data was performed to investigate white matter organisation in a large, well-defined ASD sample. Relative to neurotypical individuals, the ASD group showed significantly reduced FA in number of bilateral brain regions, some of which were associated with the social communication deficits and restricted repetitive behaviours characteristic of ASD. Additionally, an age-related white matter difference in the left anterior thalamic radiation was observed between groups indicating that the developmental trajectory of white matter is aberrant in this region in ASD. Recent methodological research has suggested that the standard TBSS approach has limitations. Advances in diffusion MRI analytic software now offer a number of alternative approaches to investigate white matter organisation in ASD including CSD based tractography and segmentation analyses. Future work will focus on isolating each of the tracts identified in this TBSS study using advanced tractography methods. This will allow for better characterisation of white matter abnormalities in these tracts as well as more in depth analyses of associations between disrupted structural connectivity and behaviour in ASD.

6 Whole Brain Analysis of Grey Matter Cortical Structure

6.1 Introduction

6.1.1 Typical Grey Matter Development

To date, whole-brain volumetric analyses have been the primary tool used to investigate neuroanatomical abnormalities in ASD (Waiter et al., 2004, Ecker et al., 2012, Toal et al., 2010, Rojas et al., 2006, McAlonan et al., 2002, Greimel et al., 2013). Acquisition of higher resolution data has shifted the focus from cortical volume to investigating the two determinants of grey matter volume, cortical thickness (CT) and surface area (SA). Additionally, a local gyrification index (GI) or degree of folding as well as a measure of sulcus depth can be derived from these cortical measures to evaluate cortical structure. Research has shown that these measures follow different developmental trajectories (Raznahan et al., 2011, Mills and Tamnes, 2014). Cortical volume follows a trajectory in neurotypical individuals in which cortical volume is at its highest in late childhood/early adolescence and then reduces with decreasing velocity before stabilising in the third decade of life (Mills and Tamnes, 2014). Cortical thickness and surface area appear to follow a similar trajectory with cortical thickness peaking earlier at approximately 8 – 9 years of age and declining more rapidly (Wierenga et al., 2014, Mills and Tamnes, 2014) than surface area. Peak gyrification occurs in early childhood and decreases linearly thereafter (Raznahan et al., 2011, Hogstrom et al., 2013). Cortical thickness and surface area measures are genetically uncorrelated, phenotypically independent and highly heritable (Winkler et al., 2010,

Panizzon et al., 2009, Sanabria-Diaz et al., 2010) indicating that minimal co-variation exists between these measures.

6.1.2 Significance of Independent Investigation of Cortical Measures

Studies of individuals with ASD have identified abnormalities in cortical grey matter (see Chapter 1, Section 1.5.1.1). Research suggests that these differences are largely driven by differences in surface area (Im et al., 2008, Winkler et al., 2010) rather than cortical thickness. This may contribute to the inconsistent differences observed across grey matter structural studies of ASD (Amaral et al., 2008, Stanfield et al., 2008, Anagnostou and Taylor, 2011).

Dendritic arborisation and pruning and/or myelination changes are believed to influence cortical thickness (Sowell et al., 2004) whereas division of progenitor cells during embryogenesis is hypothesised to drive surface area measures (Chenn and Walsh, 2002, Rakic, 1988). Disruption to genes involved in these processes are thought to contribute to ASD pathology. Cellular and animal models of autism candidate genes, such as contactin-associated protein 2 (CNTNAP2), a neurexin-related cell-adhesion molecule, have demonstrated that arborisation and dendritic spine development may be abnormal in ASD (Anderson et al., 2012). Deletion of Pten, a gene identified in ASD individuals with macrocephaly and involved in neurogenesis, was shown to accelerate differentiation of progenitor cells (Amiri et al., 2012). Thus these processes appear to be biologically distinct based on both imaging and genetic literature. Studying these cortical measures separately in ASD can provide a better understanding of the biological underpinning of cortical development in the disorder.

6.1.3 Overview of Cortical Thickness Findings

Cortical thickness is quantified by measuring the layer of tissue between the outer pial surface and the inner grey-white matter boundary (see Figure 6.1 and Section 6.3.3 for details) (Fischl and Dale, 2000). In neurotypical individuals, the thickness of the cortical mantle is both region and hemisphere specific (Kabani et al., 2001) where temporal and insular regions have the greatest cortical thickness in comparison to frontal and occipital where the thinnest cortex is found (Ribeiro et al., 2013). Abnormal cortical thickness has been identified in a number of different disorders including attention deficit hyperactivity disorder, Williams syndrome and 22q11.2 deletion syndrome [see (Dennis and Thompson, 2013) for review] and recently, a number of studies have investigated thickness of the cortex in ASD with varied results. Many of the studies to date have been underpowered (Hyde et al., 2010, Hardan et al., 2006, Hardan et al., 2009, Chung et al., 2005) with groups including 18 or fewer individuals. Furthermore, many of the experimental groups were not matched for IQ (Hardan et al., 2009, Raznahan et al., 2010, Zielinski et al., 2014, Mak-Fan et al., 2012). Studies have shown that IQ may be associated with variability in cortical thickness measures (Shaw et al., 2006, Narr et al., 2007), indicating that it may be a factor contributing to the inconsistent results. Furthermore, gender differences in developmental trajectories have been reported (Raznahan et al., 2011), thus indicating that gender is also a factor which adds heterogeneity to the samples in existing studies (Libero et al., 2014, Doyle-Thomas et al., 2013a).

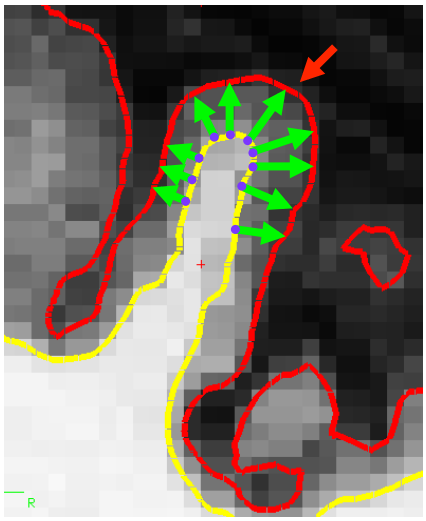

Figure 6.1. Illustration of how Cortical Thickness is Estimated

The yellow line represents the grey-white matter boundary. The red line represents the grey-matter pial boundary. The green arrows demonstrate the section of the cortex which is measured to give an estimate of cortical thickness. Taken from (http://surfer.nmr.mgh.harvard.edu)

Cortical structure changes also throughout development and therefore the age-range of subjects within studies needs to be considered as an influencing factor. Contrary to expectations, given theories of inadequate pruning in ASD, cortical thinning has been observed in the parietal, superior and ventral temporal, superior, medial and middle frontal regions during late adolescence and early adulthood relative to controls (Misaki et al., 2012, Wallace et al., 2010, Chung et al., 2005, Ecker et al., 2014). Hardan et al. (2009) found that there was an accelerated decline in cortical thickness in frontal, parietal and occipital regions in ASD compared to controls during late childhood but increased cortical thickness in the temporal lobe. Conversely, studies of ASD with mean age in adulthood indicated that cortical thickness was

greater in frontal (Hyde et al., 2010, Ecker et al., 2013, Doyle-Thomas et al., 2013a, Libero et al., 2014), parietal, temporal and occipital regions as well as the cingulate cortex (Hyde et al., 2010, Ecker et al., 2013, Doyle-Thomas et al., 2013a). These studies also demonstrated region specific cortical thinning in different areas within these regions. Between these studies, there was some discrepancy in terms of the direction of aberrant thickness. For example, Ecker et al. (2013) showed greater cortical thickness in the postcentral gyrus in ASD relative to controls whereas Hyde et al. (2010) demonstrated reduced thickness in the same brain region. Other studies of adults with ASD reported reduced cortical thickness only (Scheel et al., 2011, Hadjikhani et al., 2006) while one study found no differences in cortical thickness between groups (Doyle-Thomas et al., 2013a).

A number of studies have probed age-related changes in cortical thickness to determine if the developmental trajectory of this cortical metric differs in ASD. Scheel et al. (2011) found that the ASD group demonstrated significantly less age-related cortical thinning in frontal, parietal, temporal and occipital regions in comparison to controls. Age related differences in cortical thickness were observed in the temporal (Wallace et al., 2010, Raznahan et al., 2010), frontal (Raznahan et al., 2010, Zielinski et al., 2014, Libero et al., 2014), occipital (Zielinski et al., 2014) and right parietal regions (Misaki et al., 2012, Scheel et al., 2011, Zielinski et al., 2014), where cortical thickness in the control group decreased with age but not in the ASD group. However, Mak-Fan et al. (2012) found no age related effects of cortical thickness between groups. Zielinski et al. (2014) suggested that abnormal cortical thickness in ASD can be characterised by rapid expansion in early childhood followed by accelerated thinning in early childhood and subsequently, a slowing or reduction in cortical thinning occurs in

late adulthood, a hypothesis that appears to be somewhat corroborated by the previous studies but requires further investigation.

6.1.4 Overview of Surface Area Findings

Fewer studies have focused on surface area, gyrification and sulcus depth in ASD and no study has investigated these cortical measures in addition to cortical thickness in the same adolescent ASD population. As with studies probing cortical thinning, inconsistent results are notable across these measures. Complications arise in evaluating surface area due to the number of ways it can be defined (Ribeiro et al., 2013). Studies can quantify surface area using the grey-white matter boundary (white matter surface), the grey-pia mater surface (pial surface) or by averaging the two metrics (see Section 6.3.3 for details). The metric chosen is often not clarified. An increase in surface area was observed across temporal, frontal and parietal-occipital regions (Hazlett et al., 2011, Ecker et al., 2013) as well as the in the right cingulate cortex in ASD (Doyle-Thomas et al., 2013b). Ecker et al. (2013) also found reduced cortical surface area within these sub-regions however results reported are at an uncorrected threshold. Reduced surface area was identified in the left fusiform and middle temporal gyri (Libero et al., 2014) and the prefrontal and anterior temporal cortices (Ecker et al., 2014). Conversely, no difference in surface area was found in other studies of ASD (Raznahan et al., 2010, Mak-Fan et al., 2012, Wallace et al., 2013, Raznahan et al., 2012). Three of these studies did not have IQ matched groups (Raznahan et al., 2010, Raznahan et al., 2012, Mak-Fan et al., 2012) and the other included medicated ASD participants (Wallace et al., 2013) which may have influenced acquisition of neuroanatomical data.

6.1.5 Overview of Gyrification Findings

Cortical folding or gyrification is believed to be associated with the prenatal development of neuronal connections (Armstrong et al., 1995, Regis et al., 2005, White et al., 2010). The process and function of cortical folding are poorly understood, however one emerging hypothesis suggests that cortical shape results from underlying patterns of connectivity whereby strongly connected regions migrate towards either other to produce efficient neural networks (Van Essen, 1997, Schaer et al., 2013). Cortical folding is also believed to represent the ongoing process of myelination and synaptic remodelling (White et al., 2010). In ASD, an increased gyrification index (see Section 6.3.3 for details) was identified in children and adolescents in the left frontal cortex (Hardan et al., 2004, Jou et al., 2010), left precuneus (Wallace et al., 2013), right parietal cortex (Kates et al., 2009) and bilateral lateral occipital regions (Wallace et al., 2013) relative to controls. In contrast reduced cortical folding was observed in the right parietal, inferior frontal, precentral and parieto-occipital regions (Schaer et al., 2013) and the left supramarginal gyrus (Libero et al., 2014). Conversely, no difference in gyrification index was observed in a study by Casanova et al. (2009). Two studies have investigated the effect of age on gyrification; one did not find any age-related difference between groups in 41 adolescents with ASD (Wallace et al., 2013) and the other noted a age-related difference reduction in gyrification in the left supramarginal gyrus in the ASD group relative to the control group in a cohort of 55 children with ASD (Libero et al., 2014) although this finding did not survive co-variation with intracranial volume. Together, these findings demonstrate that a distinct pattern of disrupted cortical folding has yet to be established in ASD.

6.1.6 Overview of Sulcal Findings

Sulcus anatomy is the most infrequently investigated cortical measure in ASD. Sulcal depth was examined in the current study (see Section 6.3.3 for details). One previous study identified deeper sulci in the bilateral parietal operculum and bilateral intraparietal sulcus in individuals with high functioning autism and Asperger's syndrome respectively (Nordahl et al., 2007) with an age-related reduction in sulcus depth in the left intraparietal sulcus in the Asperger's group. Abnormality in sulcus formation in terms of either sulcal depth, length or position was observed in central, parietal, and frontal medial sulci (Auzias et al., 2014), left insula and right intraparietal sulcus (Shokouhi et al., 2012) and bilateral superior frontal sulci, left inferior sulcus and the right Sylvian fissure in ASD (Levitt et al., 2003). In the study by Auzias et al. (2014) the trajectory of sulcal abnormality differed significantly between groups in a population of 59 ASD and 14 typically developing children aged 18 – 108 months where the control group was significantly correlated with age but the ASD group was not. The low number of control participants may have impacted results from this study. Further investigation is required to understand the role of sulcal formation in ASD pathology.

6.1.7 Cortical Measures and their Association with Clinical Symptoms

A limited number of studies have attempted to relate the findings of cortical abnormalities in ASD to clinically relevant factors. Reduced cortical thickness was associated with deficits measured by the ADI-R in reciprocal socio-emotional interaction in the frontal lobe and with restricted repetitive behaviours in the temporal

lobe in ASD (Hardan et al., 2009) in pre-pubescence. Conversely, in adults, an increase in cortical thickness in the dorsolateral frontal region was correlated with greater ADI-R scores indicating increased deficit in the communication and restricted repetitive behaviours domain (Ecker et al., 2013) while thicker rostral cingulate cortex and reduced cortical thickness in the left orbitofrontal gyrus were associated with greater impairment on the ADI-R social domain (Doyle-Thomas et al., 2013a). Doyle-Thomas et al. (2013b) indicated that greater surface area in the right insula and the left isthmus was related to greater impairment based on ADI-R and ADOS-G scores in the social domain respectively in a group with a mean-age in adulthood. In adolescence, reduced gyrification in the occipital region in the ASD group was associated with greater total ADI-R, social interaction and communication sub-scale scores (Schaer et al., 2013). Greater sulcus depth in the right intraparietal sulcus in adolescents aged 7.5 – 18 years (Nordahl et al., 2007) and in the left central sulcus in children aged 1.5 – 9 years (Auzias et al., 2014) was correlated with social deficits measured by the ADOS. Conversely, reduced sulcus depth was associated greater ADOS and CARS scores in the right intraparietal sulcus (Auzias et al., 2014). Furthermore, deeper left intraparietal (Nordahl et al., 2007) and left central sulci (Auzias et al., 2014) was associated with repetitive behaviours measured by the ADOS.

Collectively, research to date indicates that cortical abnormalities in ASD are region specific and also dependent on the stage of development and level of ability of the participants.

6.1.8 Aims

The main objectives of this study are to:

1. Investigate cortical thickness, surface area, gyrification index and sulcus depth in a well-defined large sample of individuals with ASD and age and IQ-matched neurotypical controls.
2. Evaluate age-related changes in cortical metrics between the ASD and control groups.
3. Determine if regions that differ between groups are associated with clinical deficits in ASD.

6.2 Methods

6.2.1 Participants

Participants for the study were taken from the 'McGrath', 'Delmonte' and 'Fitzgerald' cohorts. Details of the participants for this study (study 4) are described in Table 6.1 and in Chapter 2, Section 2.2. Distribution of social and communication deficits and restricted repetitive behaviours are illustrated in Figures 6.2 and 6.3 respectively. Tests of normal distribution of these behavioural measures are reported in Table 6.2.

Table 6.1. Participant Demographics

	Control	**ASD**	**P-value**
Number	63	63	
Gender	Male	Male	
Mean age ± SD; range	16.34 ± 3.06; 10.25-24.8	15.92 ± 3.48; 10-25.9	0.48
Mean Full Scale IQ ± SD; range	111 ± 12.03; 81-132	106.56 ± 15.7; 72-145	0.077
Social Communication Deficits		24.87 ± 6.86: 12-40	
Restricted Repetitive Behaviours		4.83 ± 2.46: 0-12	
ADOS: Social and Communication		10.21 ± 2.835; 7-19	

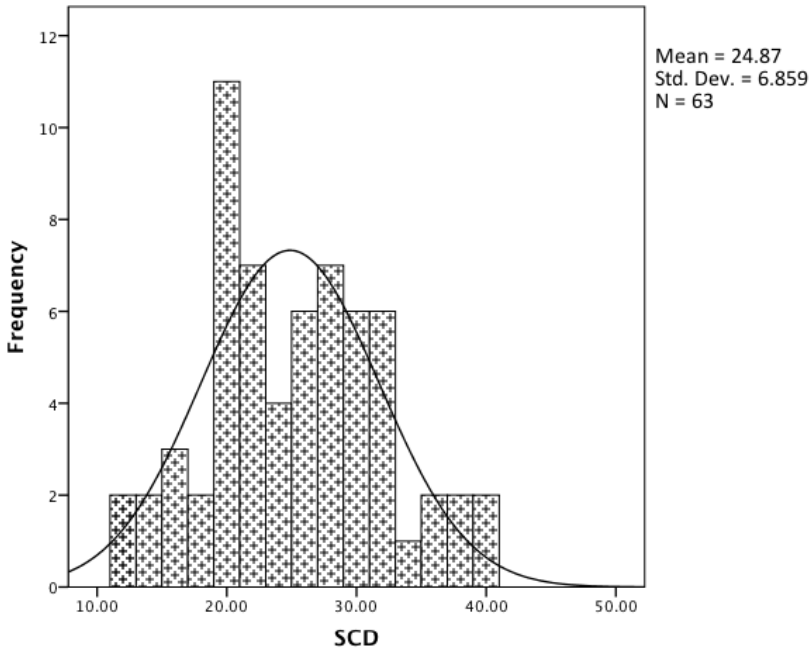

Figure 6.2. Distribution of Social and Communication Deficits Domain Scores

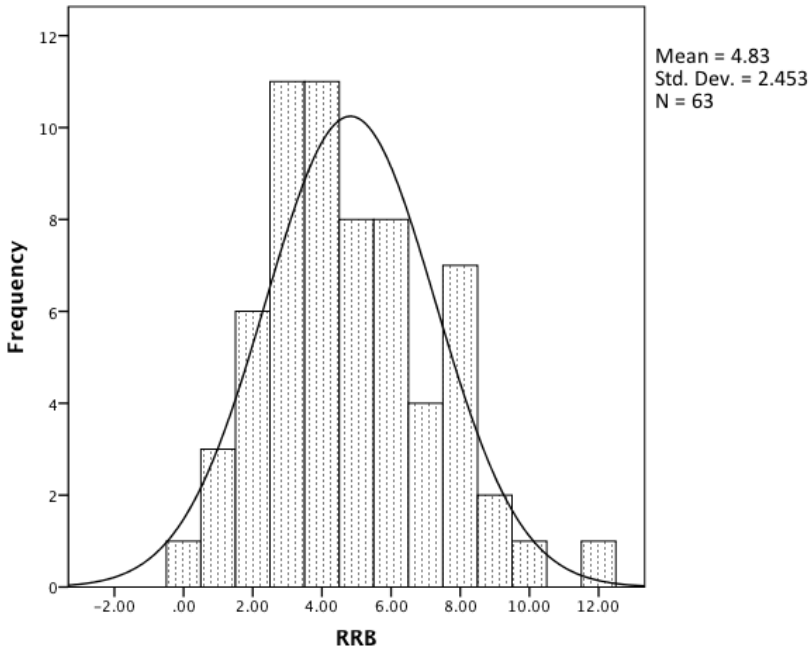

Figure 6.3. Distribution of Restricted and Repetitive Behaviours Domain Scores

Table 6.2. Tests of Normal Distribution of Behavioural Measures

Behavioural Measure	Shapiro-Wilk Statistic	Shapiro-Wilk P-Value	Skew	Kurtosis
Social and Communication Deficits	0.958	0.106	0.191	-0.526
Restricted and Repetitive Behaviours	0.962	0.142	0.5	0.31

6.2.2 MRI Preprocessing

A high-resolution T1-weighted image was acquired (see Chapter 2, Section 2.5.1 for details). Freesurfer image analysis software, version 5.3.0 (http://surfer.nmr.mgh.harvard.edu) was used to generate a cortical surface model to provide measures of volume, cortical thickness, surface area, gyrification and sulcus depth at each vertex (Fischl et al., 1999, Dale et al., 1999, Fischl et al., 2004, Fischl and Dale, 2000). Preprocessing involved a multi-step semi-automated pipeline. First, visual inspection of individual subject data for motion artifacts was completed. Images were then transformed using 12 degrees of freedom affine Talairach transformation into MNI space. Intensity normalisation for magnetic field inhomogeneity correction and removal of non-brain tissues (skull stripping) was performed. Automated segmentation of the white matter surface and reconstruction of the pial surface was carried out. The surface models were then visually inspected and manually edited where necessary to correct for inaccuracies in generating the grey-white matter boundary.

6.2.3 Grey Matter Cortical Measures

Cortical thickness was measured by calculating the distance between the grey-white matter boundary and the pial surface (Figure 6.1). Surface area at each vertex

was calculated by averaging the area of the triangles adjacent to each vertex. The grey-pial boundary was selected for surface area analysis (https://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferSupport). To measure the local gyrification index, a spherical 25mm region of interest is generated at each vertex and a ratio of the convoluted inner pial surface to the outer hull layer is calculated. Sulcus depth was defined by generating a ratio between the area of the sulcus and the area of the convex hull (outer curved region of the sulcus), which results in outward structures yielding a positive sign (sulci) and inward structures yielding a negative sign (gyri).

6.2.4 Statistical Analyses

Vertex-by-vertex group differences were evaluated using QDEC, an application within the Freesurfer software package. Cortical maps were smoothed with a 10mm full-width-half-Gaussian kernel. As gyrification data is already smoothed within the preprocessing steps, a 5mm full-width-half-Gaussian kernel was applied to prevent type II errors. To correct for multiple comparisons, a Monte Carlo simulation with 10,000 iterations was performed with a clusterwise threshold of $p < 0.05$. To investigate the effects of age on cortical measures, age and IQ were demeaned and included as variables in the analysis model. For all significant clusters, individual measures were extracted and exploratory Spearman correlations with clinical measures of SCD and RRB were carried out. Bonferroni correction of correlation analyses were performed based on the number of clinical measures used ($p = 0.05/2 = 0.025$). Total intracranial volume, mean thickness and mean surface area were demeaned and added as nuisance variables where appropriate to control for any possible influential effects of these factors.

6.3 Results

There were no significant group differences on any global cortical measure (Table 6.3). Results for between-group differences in cortical measures of grey matter including cortical thickness, surface area, gyrification and sulcus depth are described below (Figures 6.4 – 6.7 and Table 6.4).

Table 6.3. Between-Group Differences in Global Grey Matter Cortical Measures

	Control	ASD	P-value
Total Grey Matter	562257 ± 68191	562257 ± 68191	0.381
Total White Matter	530267 ± 53110	513688 ± 71655	0.143
Total Intracranial Volume	1274583 ± 10197	1279354 ± 136093	0.824
Total CSF	1008.6 ± 251.7	1028.5 ±262.6	0.665
Total Cortical Thickness	2.649 ± 0.13	2.625 ± 0.158	0.342
Total Surface Area	96471.3 ± 7437.9	95037.5 ± 11077	0.396

6.3.1 Cortical Thickness Findings

The ASD group showed reduced cortical thickness in the left hemisphere in the supramarginal ($F(1, 124) = 14.987$, $p = 0.00$), superior frontal ($F(1, 124) = 11.563$, $p = 0.002$) and precentral gyri ($F(1, 124) = 10.304$, $p = 0.002$) relative to the control group (Figure 6.4). There was increased cortical thickness in the ASD group in the left cuneus

(F (1, 124) = 12.927, p = 0.00) in addition to the right pericalcarine (F (1, 124) = 9.779, p = 0.002) and lingual gyri (F (1, 124) = 11.719, p = 0.001) in comparison to the controls.

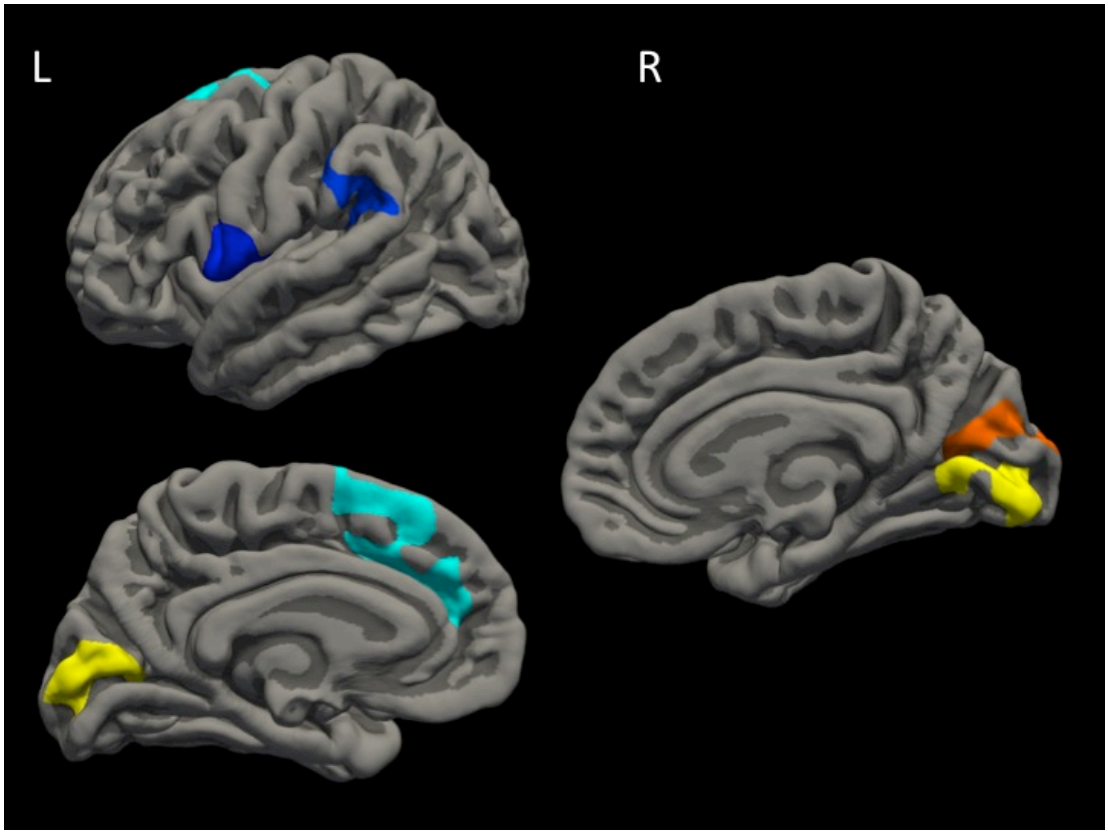

Figure 6.4 Between-Group Differences in Cortical Thickness

Greater cortical thickness in the ASD group is shown in yellow (t-value > 4)/orange (t-value > 3.5), reduced cortical thickness in ASD is shown in dark blue (t-value > 2.62) /light blue (t-value > 4), corrected for multiple comparisons, p < 0.05. L = Left hemisphere, R = Right hemisphere

6.3.2 Surface Area Findings

Greater surface area was observed bilaterally in the superior frontal (Left; (F (1, 124) = 18.647, p = 0.00; Right; (F (1, 124) = 20.119, p = 0.00) and precentral gyri (Left;

(F (1, 124) = 15.637, p = 0.00); Right; (F (1, 124) = 13.09, p = 0.00) in the ASD group when compared with the control group (Figure 6.5). The ASD group also showed an increase in surface area in the left inferior temporal gyrus (F (1, 124) = 16.630, p = 0.00) and the precuneus (F (1, 124) = 13.359, p = 0.00). Laterally, in the right hemisphere, the ASD group revealed larger surface area in the superior temporal (F (1, 124) = 16.987, p = 0.00) and lateral occipital regions (F (1, 124) = 14.410, p = 0.00) relative to controls.

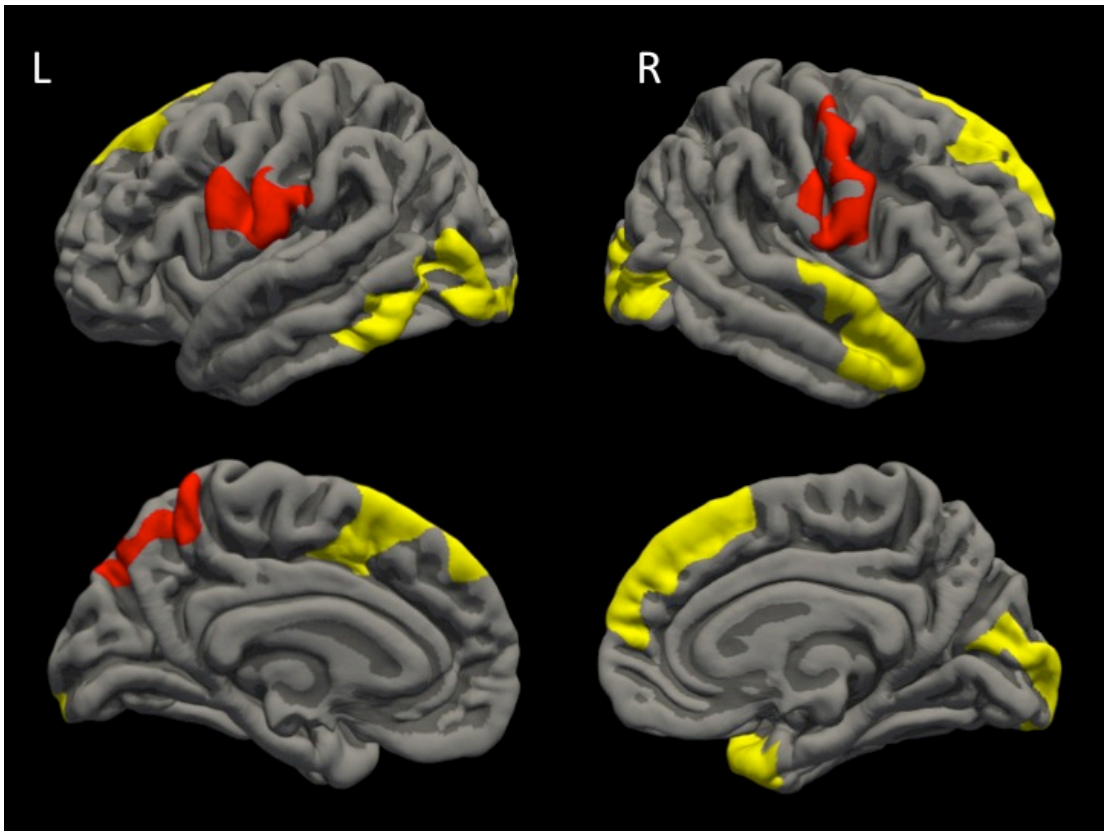

Figure 6.5. Between-Group Differences in Surface Area

Greater surface area in the ASD group is shown in yellow (t-value > 4)/red (t-value > 2.62), corrected for multiple comparisons, p < 0.05. L = Left hemisphere, R = Right hemisphere.

6.3.3 Gyrification Index Findings

The only between-group difference in gyrification was observed in the right inferior temporal region (F (1, 124) = 8.741, p = 0.004) (Figure 6.6) where the ASD group had increased cortical folding in comparison to the control group.

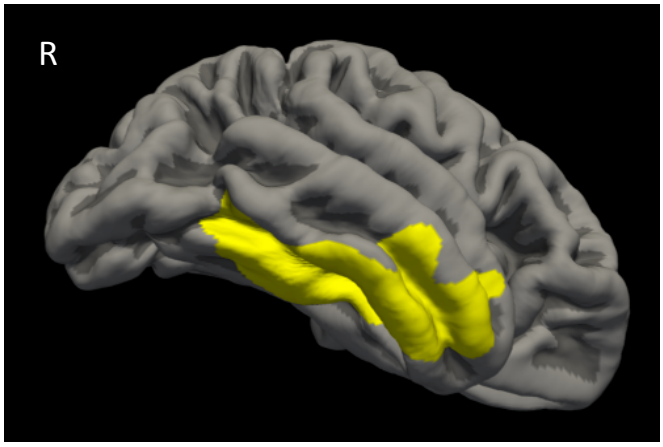

Figure 6.6. Between-Group Differences in Gyrification

Greater gyrification in the ASD group is shown in yellow, corrected for multiple comparisons, $p < 0.05$. L = Left hemisphere, R = Right hemisphere

6.3.4 Sulcus Depth Findings

Reduced sulcus depth was observed in the right rostral anterior cingulate (F (1, 124) = 19.234, p = 0.004) and the right insula (F (1, 124) = 8.633, p = 0.00) in the ASD group relative to the control group (Figure 6.7).

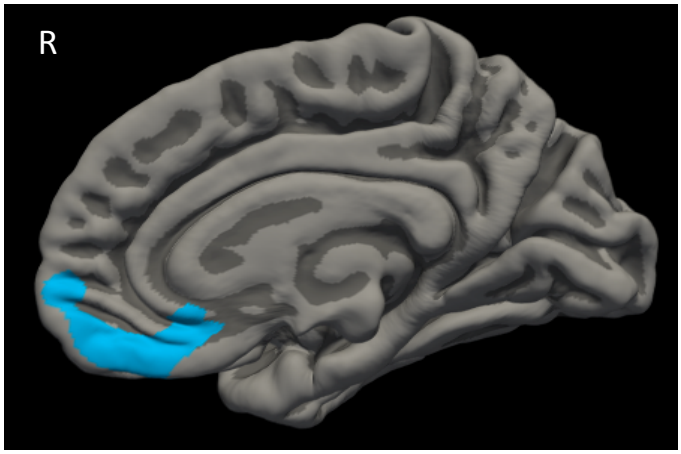

Figure 6.7. Between-Group Differences in Sulcus Depth

Reduced sulcus depth in ASD is shown in blue, corrected for multiple comparisons, p < 0.05. L = Left hemisphere, R = Right hemisphere

Table 6.4. Group Differences in Grey Matter Cortical Measures

	Region/Gyrus	Size (mm^2)	X	Y	Z	Direction
	Cortical Thickness (mm)					
L	Supramarginal	932.91	-52.7	-46.6	27	ASD < CTRL
L	Cuneus	1071	-3.8	-87	12.6	CTRL < ASD
L	Superior Frontal	1518.37	-12	22.4	34.5	ASD < CTRL
L	Precentral	854.01	-57	1.6	7.8	ASD < CTRL
R	Lingual	954.18	5.6	-89.9	-9.4	CTRL < ASD
R	Percalcarine	881.07	15.6	-67.3	9.7	CTRL < ASD
	Surface Area (mm^2)					
L	Superior Frontal	1636.72	-7.7	45.3	44.1	CTRL < ASD
L	Precentral	1232.65	-58.9	4.9	21.6	CTRL < ASD
L	Inferior Temporal	2574.03	-55.3	-42.9	-18.2	CTRL < ASD
L	Precuneus	977.86	-10.4	-61.1	54.3	CTRL < ASD
R	Superior Frontal	2211.09	7	39.1	45.5	CTRL < ASD
R	Superior Temporal	1979.12	48.2	-7.1	-13.2	CTRL < ASD
R	Lateral Occipital	2477.61	42.2	-83.5	-1.1	CTRL < ASD
R	Precentral	1037.67	52.8	6.4	30.4	CTRL < ASD
	Gyrification Index					
R	Inferior Temporal	3002.78	46.4	-5.4	-35.4	ASD < CTRL
	Sulcus Depth Ratio					
R	Anterior Cingulate	849.27	6.1	24.4	-9.5	ASD < CTRL
R	Insula	920.79	35.6	-4.3	13.1	ASD < CTRL

All reported regions are corrected for multiple comparisons, $p < 0.05$. X, Y, Z = Peak Montreal Neurological Institute (MNI) Co-ordinates, L = Left, R = Right, SD = Standard Deviation.

6.3.5 Group-by-Age Interaction with Cortical Measures

Gyrification was the only cortical measure that revealed a significant group-by-age interaction in the superior frontal gyrus (MNI: -10 64.3 6.5; p = 0.001) (Figure 6.8). The control group showed a significant decrease in gyrification with age (r = -0.317, p = 0.014) whereas the ASD group did not show any significant change (r = 0.06, p = 0.64). All between-group differences across cortical measures revealed a significant negative correlation with age in both groups (p < 0.05) with the exception of surface area in the left precuneus gyrus and the right superior temporal region, which showed no correlation in either group.

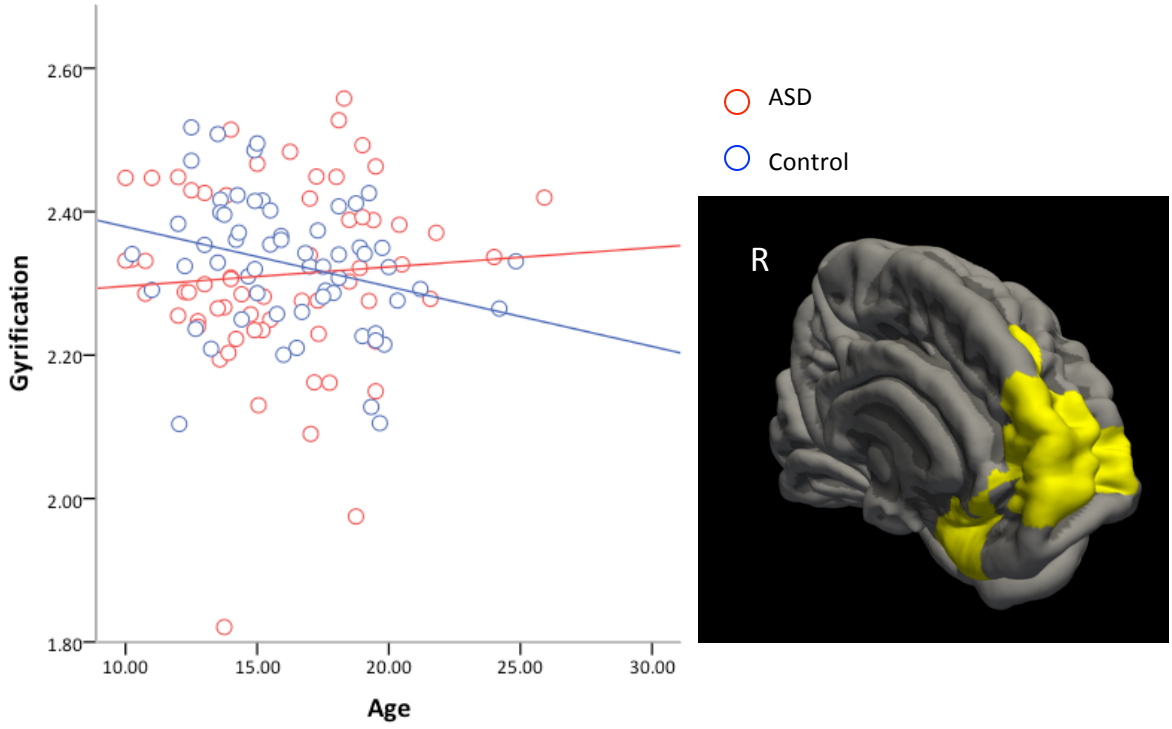

Figure 6.8. Group-by-age Interaction Effect of Gyrification in the Right Superior Frontal Gyrus (SFG)

The region of the right SFG showing a group-by-age interaction effect in gyrification index is shown in yellow, corrected for multiple comparisons, p < 0.05, R = Right.

6.3.6 Correlation Analyses

Greater social and communication deficits were correlated with reduced cortical thickness in the left superior frontal gyrus ($r = -0.347$, $p = 0.005$) in the ASD group. This was the only association that survived Bonferroni correction. A number of other of regions demonstrated an uncorrected association with clinical measures. There was a correlation between cortical thickness and the left precentral gyrus ($r = -0.269$, $p = 0.033$) in the ASD group. Reduced cortical thickness was also associated with greater social and communication difficulties (SCD) in this region. Sulcus depth in the right insula was negatively correlated with the SCD domain ($r = -0.252$, $p = 0.047$) indicating that reduced sulcus depth was associated with greater social impairments. None of the cortical measures were significantly associated with the fixed interests and restricted behaviours domain.

6.4 Discussion

6.4.1 Overview of Findings

The objective of this study was to investigate the composite cortical measures of thickness and surface-area as well as their derived metrics, gyrification and sulcus depth, in a well-defined sample of ASD participants relative to controls. This is the first study to investigate these four measures in an adolescent ASD population. Findings showed that although individuals with ASD have comparable global estimates of volume, thickness and area to controls, they demonstrated region specific differences. Reduced cortical thickness in frontal and parietal regions and greater thickness in occipital regions was observed in the ASD group. Relative to controls, the ASD group also showed greater surface area in bilateral frontal and temporal regions, the right precuneus and left lateral occipital region. Between-group differences in cortical folding were characterised by greater gyrification in the right inferior temporal region and reduced sulcal depth in the right anterior cingulate and insula. An age-related difference in gyrification was also identified in the right superior frontal region. Collectively, these findings corroborated previous results and add to current literature that indicates that cortical development is aberrant in ASD.

6.4.2 Atypical Cortical Thickness in ASD

Our sample size had a mean age in adolescence, the period at which an increase in cortical thinning is thought to occur, possibly due to synaptic elimination, dendritic arborisation and intra-cortical myelination (Sowell et al., 2004, Raznahan et al., 2011, Mills and Tamnes, 2014, Wierenga et al., 2014). Results from the present study

revealed greater cortical thinning in the left supramarginal, precentral and superior frontal gyri in the ASD group relative to controls. These findings support previous studies which identified cortical thinning in a similar age group (Misaki et al., 2012, Wallace et al., 2010, Chung et al., 2005) however only the finding of increased cortical thinning in the supramarginal gyrus overlapped with a previous study (Wallace et al., 2010). These regions displaying aberrant cortical thickness have previously been implicated in ASD pathology. The left supramarginal gyrus has been associated with language processing deficits (Stefanatos and Baron, 2011) and reduced grey matter in the left precentral gyrus has been linked with social and communication deficits in ASD (Hadjikhani et al., 2006). Interestingly, in the current study, reduced cortical thickness in the left superior frontal gyrus, which is implicated in higher cognitive function and working memory, was also associated with greater social and communication deficits further supporting the hypothesis that aberrant cortical development may underlie the behavioural deficits observed in individuals with ASD. Moreover, given the role of frontal regions in executive function (Hill, 2004), the cortical thinning in the ASD group in the left superior frontal gyrus may offer insight into the neurobiological abnormalities underpinning executive dysfunction in ASD.

Evidence has suggested that genetic variation within ASD individuals may also influence cortical metric differences. Reduced cortical thickness in bilateral superior middle temporal gyri, pre- and postcentral gyri, the anterior cingulate and in the right fronto-polar cortex in neurotypical individuals was found to be associated with the MET proto-oncogene gene variation (C allele), which has been associated with ASD in genetic studies (Hedrick et al., 2012).

Results from the current study also demonstrated an increase in cortical thickness bilaterally across occipital regions indicating that aberrant cortical development in ASD is not uniform across brain regions. Significantly, the developmental trajectory of grey matter has been shown to differ across brain regions. A longitudinal study of neurotypical individuals by Sowell et al. (2004) found cortical thinning in frontal, parietal and occipital regions but an increase in cortical thickness in temporal regions, possibly due to consolidation of language ability. Similar region specific differences in cortical thickness were observed in a subsequent study (Shaw et al., 2008). These studies highlight the significance of age and brain region when interpreting differences in cortical thickness across studies. Therefore, the increased cortical thickness observed in occipital regions may represent an altered developmental trajectory specifically within these regions in ASD. This may also be representative of the enhanced visuo-spatial processing that has been consistently reported in ASD (Caron et al., 2006, Caron et al., 2004, Jolliffe and Baron-Cohen, 1997, Joseph et al., 2009).

6.4.3 Greater Surface Area in ASD

In this study, greater surface area was identified in the ASD group in a number of regions including bilateral superior frontal, precentral and temporal gyri, left precuneus and the right lateral occipital region. Another study also demonstrated greater surface area in a number of regions including the bilateral superior temporal and parahippocampal gyri, left middle frontal gyrus and the right precentral and supramarginal gyri (Ecker et al., 2013). This same study identified reduced surface area in the bilateral dorsolateral prefrontal cortex, orbitofrontal and superior frontal gyri in

addition to temporal and parietal regions. An adult cohort was examined in this study which may contribute to the discrepancy in results. The neuroanatomical regions that demonstrated aberrant surface area in the present study have been associated with functional deficits in ASD. For example, the bilateral temporal gyri have been implicated in impaired social perception (Ashwin et al., 2007) and social cognition (Silani et al., 2008) in ASD. Notably, a number of brain regions including the superior frontal and precentral gyri as well as occipital regions showed differences in both cortical thickness and surface area. This demonstrates that brain regions are susceptible to disruption of both distinct neurobiological mechanisms, namely aberrant cortical thickness and surface area development, each of which may equally contribute to ASD symptomology. Genetic variance has also been shown to affect surface area estimates in ASD. Increased surface area in the posterior cingulate, caudal anterior cingulate, rostral middle frontal and lateral orbitofrontal, par orbitalis and pars triangularis was previously associated with presence of the BDNF met allele in ASD (Raznahan et al., 2009).

6.4.4 Supporting Evidence from Neuropathological Studies – Minicolumn Pathology

Post-mortem studies of cortical tissue of ASD provide support for the current findings and offer a possible mechanism by which aberrant cortical development occurs. The 'radial unit hypothesis' is a theory relating to the cellular underpinnings of surface area and cortical thickness. This theory postulates that surface area is be driven by the number of minicolumns, neuronal subunits within the cortex, while cortical thickness is driven by the number of cells within a column (Rakic, 1988). Post-

mortem studies found a greater number of minicolumns in the frontal cortex in ASD brains (Casanova et al., 2006, Buxhoeveden et al., 2006) suggesting that the atypical surface area in frontal regions may be due to a larger number of minicolumns in that region. These studies also identified narrowed minicolumns in post-mortem child and adult ASD brains, which also provides a possible pathophysiological mechanism underlying the finding of reduced cortical thickness, particularly in frontal regions, in this study. Furthermore, these findings may reflect a disruption to cortical cytoarchitecture during development, which may subsequently lead to disrupted cortical connectivity (Casanova and Trippe, 2009). Minicolumns are considered to act as a template to guide neuronal pathway and circuit organisation (Casanova et al., 2002b, Casanova et al., 2002a). Casanova and colleagues postulate that a systematic disruption of the coordination of neocortex development due to abnormal minicolumar structure is key to understanding the pathology of abnormal cortical connectivity in ASD, a theory that requires further investigation (Casanova and Trippe, 2009).

6.4.5 Abnormal Gyrification Indices

In this study, the ASD group had greater gyrification in the right inferior temporal region relative to controls and an age-related difference in gyrification was observed in the right superior frontal region where gyrification declined with age in the control group but there was no reduction in gyrification in the ASD group. These findings suggest that cortical development is abnormal in ASD. The finding of increased gyrification is consistent with a number of previous studies (Wallace et al., 2013, Kates et al., 2009, Jou et al., 2010, Hardan et al., 2004). Cellular studies have shown that

increased cortical folding and a high gyrification index is associated with an abundance of basal and bipolar radial glial cells respectively (Borrell and Gotz, 2014) and the Van Essen (1997) model of convolution development postulates that disrupted gyrification reflects aberrant structural connectivity due to tension applied by radial glial cells during neuronal development. This theory suggests that strongly connected regions migrate towards each other forming gyri while weakly connected regions drift apart forming sulci. A more recent study suggests that cortical folding is driven by 'pushing' of wiring axons due to the majority of axon fibers connecting to gyral rather than sulcal regions (Nie et al., 2012). Nie and colleagues demonstrated that distributions of fiber endings points closely follow gyral folding patterns and concentrate on gyral regions. A study investigating both gyrification and functional connectivity (the co-ordination of processing between brain regions) found that greater frontal gyrification was associated with greater short range connectivity within the frontal lobe (Schaer et al., 2013) thus offering neuroanatomical and functional support for these theories. There was no association between disrupted long-range connectivity and regions of aberrant gyrification. Therefore, the increased gyrification observed in the inferior temporal and superior frontal regions in this study may reflect a mechanism adapted by individuals with ASD to generate stronger neural connectivity in these regions following disrupted neuronal development.

Damage to right temporal lobe can result in impaired facial recognition (de Schonen et al., 2005) and difficulty processing facial expressions (Ahs et al., 2014). Difficulty distinguishing facial expressions is a common characteristic reported in ASD (Lozier et al., 2014), therefore it is possible that aberrant gyrification in this region may

contribute to the abnormal face processing that has been so widely reported in the ASD population.

6.4.6 Aberrant Sulcus Depth in ASD

The reduced sulcus depth in the ASD group in the right anterior cingulate and insula is a finding that has not previously been reported, but there are only a handful of studies investigating sulcus formation in ASD and these have been small (Nordahl et al., 2007, Shokouhi et al., 2012) or performed in young children (Auzias et al., 2014). The anterior cingulate and insula were two brain regions activated during neurotypical attention orienting in ASD (Chapter 3, Section 3.4.3). The anterior cingulate has a critical role in decision making, reward anticipation, empathy and emotion as well as facilitating modulation of the ventral attention network. In a smaller sub-sample of this cohort, the ASD group demonstrated stronger functional connectivity between the anterior cingulate and the neural correlates of the ventral attention network relative to controls. Van Essen's theory (Van Essen, 1997) would suggest that the reduced sulcus depth in the right anterior cingulate and insula may represent an attempt to strengthen cortical connectivity in the ASD group in these regions. It is possible that abnormal sulcal depth is related to aberrant functional connectivity, and this would be an interesting avenue for future research. Recent advances in neuroimaging analytic methods will allow researchers to perform integrated analyses of sulcus depth and functional connectivity. Genetic factors also influence sulcal formation, however no studies have investigated this link in an ASD population. Monozygotic twins were significantly more similar in their representations of neocortical sulci (Lohmann et al., 1999) and McKay et al. (2013) showed that sulcus depth and position of the central

sulcus is a genetically mediated trait. This indicates that, as for the other cortical measures discussed above, genetic variation can impact cortical folding and sulcal formation.

6.4.7 Multiple Cortical Abnormalities in the Superior Frontal Region

In this study, there were between-group abnormalities of cortical thickness and surface area as well as significant between-group age-related difference in gyrification in superior frontal regions in the ASD group. It is possible that such cortical abnormalities may underlie the increased grey matter volume that has been reported in frontal regions (Giorgio et al., 2010, Girgis et al., 2007, Toal et al., 2010, Mak-Fan et al., 2012). Increased grey matter volume has been associated with more severe restricted and repetitive behaviours in ASD (Ecker et al., 2012) and it is known that frontal lobe lesions lead to executive dysfunction behaviours such as lack of impulse control, cognitive inflexibility, insistence for sameness and repetitive behaviours (Hill, 2004). Understanding the neurobiological pathways involved in cortical thickness, surface area and gyrification formation particularly in frontal regions may enhance our understanding of the executive dysfunction characteristic of ASD.

6.4.8 Limitations

It is possible that a lack of statistical power, due to the number of variables explored may have hindered results. Given that cortical development changes across the lifespan, it is important to highlight that this study was performed in an adolescent cohort of high functioning individuals, and it is not possible to generalise results to older or younger populations or to those with lower IQ.

6.4.9 Conclusion

In conclusion, this study demonstrated that cortical thickness, surface area, gyrification and sulcal depth formation are disrupted in a large well-defined ASD cohort. In ASD, reduced cortical thickness and an increase in surface area and gyrification was largely observed in fronto-parietal regions while increased cortical thickness and surface area was identified in occipital regions. Greater surface area and gyrification was also demonstrated in temporal regions while reduced sulcal depth was found in the right anterior cingulate and insula in ASD relative to controls. Previous literature has identified a link between risk genes for ASD and abnormal cortical development thus imaging-genetic approaches are crucial to further elucidate ASD pathology. This approach has the potential to identify biomarkers for ASD which may be used for diagnosis but more importantly as targets for future therapies.

7 Discussion

7.1 Introduction

This chapter provides an overview of the findings from each of the experimental chapters and their contributions to the current literature. Finally, the limitations of the current studies in addition to possible opportunities for future research are discussed.

7.2 Review of Aims and Results

This thesis sought to answer several questions in relation to brain structure, function and connectivity in autism spectrum disorder. Using a number of advanced neuroimaging methods, functional connectivity, structural connectivity and cortical integrity were investigated in individuals with ASD. Firstly, functional connectivity of the dorsal and ventral attention networks during an attention task was explored. Secondly, structural connectivity of the superior longitudinal fasciculus was assessed using constrained spherical deconvolution (CSD) tractography. Thirdly, whole brain voxel-wise structural connectivity was assessed by tract-based spatial statistics (TBSS). Fourthly, grey matter measures of cortical thickness, surface area, gyrification and sulcal depth were examined. The developmental trajectory of the cortical components of grey matter and white matter were also explored in ASD relative to typically developing individuals. Finally, exploratory correlation analyses were performed to assess the relationship between behavioural characteristics, social and communication deficits (SCD) and restricted repetitive behaviours (RRB) and grey and white matter structural abnormalities in ASD.

7.2.1 Overview of Findings from Chapter 3

In chapter 3, functional connectivity of the dorsal and ventral attention networks during attention orienting was investigated between 21 individuals with ASD and 21 age and IQ matched typically developing individuals. It was found that despite similar response times and functional MRI activation, there were three key between-group differences in functional connectivity. Firstly, in the dorsal attention network (DAN), relative to controls, the ASD group showed significantly weaker functional connectivity between ROIs and connected regions. Secondly, in the ventral attention network (VAN), during invalid trials, the ASD group showed stronger positive functional connectivity whilst the control group showed stronger negative functional connectivity. Finally, in the VAN, during valid trials, the ASD group showed a mixed pattern of results. Whilst the control group showed strong negative functional connectivity, the ASD group showed strong positive functional connectivity between some areas but weak negative functional connectivity between other regions of the VAN. The weaker functional connectivity shown by the ASD group in the DAN suggests weaker coherence between these brain areas while the strong positive functional connectivity exhibited by the ASD group in the VAN during the invalid trials suggests that individuals with ASD may generate compensatory mechanisms to achieve neurotypical behaviour, possibly reflected by the comparative behavioural performance on the task for both ASD and controls.

7.2.2 Overview of Findings from Chapter 4

In chapter four, the results of a study aiming to isolate three branches of the superior longitudinal fasciculus are reported in a population of 45 ASD participants and

45 controls. The novel analysis performed in this study used constrained spherical deconvolution (CSD)-based tractography to investigate the organisation organisation of white matter microstructure in three branches of the superior longitudinal fasciculus (SLF) in an ASD population. Results indicated that white matter organisation is disrupted in the left SLF I and the right SLF II. Greater FA and CL were observed in the ASD group relative to controls in the right SLF II while greater CL and a trend towards greater FA were identified in the left SLF I. Furthermore, in the ASD cohort, greater rightward asymmetry of FA and trend towards greater CL in the right SLF II was demonstrated when compared with the control group. Exploratory tract segmentation analyses illustrated that differences in white matter microstructure in these tracts were driven by increased FA, increased AD and in some segments, reduced RD along the majority of the tracts.

7.2.3 Overview of Findings from Chapter 5

In chapter 5, a whole-brain analysis of white matter organisation used the methodological approach TBSS to investigate between group differences in white matter microstructure in a large, well-defined cohort of 45 ASD and 45 matched control participants. This study demonstrated that white matter microstructure was abnormal in ASD. Widespread bilateral white matter anomalies were observed in the genu, splenium and body of the corpus callosum, anterior thalamic radiation, posterior and anterior corona radiata, right superior longitudinal fasciculus, right forceps major and minor, left cingulum and the left inferior longitudinal fasciculus. In addition, age-related atypical white matter development was demonstrated in the left anterior thalamic radiation. Exploratory correlation analyses indicated that disrupted white

matter organisation in the bilateral body of the corpus callosum and the left anterior corona radiata was associated with social and communication deficits while aberrant white matter structure in the right body of the corpus callosum and the right SLF was linked with restricted repetitive behaviours in ASD.

7.2.4 Overview of Findings from Chapter 6

In chapter 6, cortical thickness and surface area in addition to their related measures, gyrification and sulcal depth were evaluated in 63 ASD and 63 neurotypical individuals, the largest sample of adolescents studied to date. This is the first study to investigate cortical thickness, surface area, gyrification and sulcal depth in the same adolescent ASD population. Results demonstrated that although individuals with ASD have comparable global estimates of volume, thickness and area to controls, they demonstrated region specific differences in each of the cortical measures studied. Reduced cortical thickness in frontal and parietal regions and greater thickness in occipital regions was observed in the ASD group. Relative to controls, the ASD group showed greater surface area in bilateral frontal and temporal regions, the right precuneus and left lateral occipital region. Between-group differences in cortical folding were characterised by greater gyrification in the right inferior temporal region and reduced sulcal depth in the right anterior cingulate and insula. Furthermore, age-related differences in gyrification were also identified in the right superior frontal region while reduced thickness in the left superior frontal region was weakly associated with social impairment.

7.2.5 Contribution of Findings to Current Literature

7.2.5.1 Disrupted Functional Connectivity of DAN and VAN during Attention Orienting in ASD

Results of the functional connectivity analyses support the theory of abnormal cortical connectivity in autism and demonstrate that individuals with ASD may engage different neural mechanisms to a neurotypical population to perform attention orienting. The reduced functional connectivity described in the dorsal attention network (DAN) between parietal and temporal regions provides a neurobiological mechanism for a reduced ability in ASD to disengage and shift attention in a voluntary goal-driven manner which has previously been demonstrated in behavioural paradigms (Ristic and Kingstone, 2005, Wainwright-Sharp and Bryson, 1993). Findings of stronger functional connectivity in the ventral attention network (VAN) imply that individuals with ASD may implement different mechanisms to achieve successful attention orienting. Results indicate that the anterior cingulate and several other frontal regions may act to facilitate comparable behavioural performance to typically developing individuals. These are considered key regions for cognitive control (Corbetta et al., 2008, Fan et al., 2012, Matsumoto and Tanaka, 2004) suggesting that ASD individuals required stronger cognitive control input to orient attention relative to controls. Furthermore, the ASD group demonstrated reduced suppression of the superior and inferior parietal lobules in order to modulate VAN during attention orienting. Collectively, results highlight that the ASD group require greater modulation and input of key neural regions to achieve successful attention orienting. As attention orienting is considered to be a fundamental facet of joint attention, it is conceivable

that neural abnormalities in the basic cognitive function of attention orienting may be a factor in the joint attention and social communication deficits observed in ASD.

7.2.5.2 Abnormal Structural Connectivity of the Superior Longitudinal Fasciculus in ASD

Abnormal structural connectivity of the SLF was described thus further substantiating the theory of disrupted cortical connectivity in ASD. Aberrant structural connectivity was observed in the left SLF I, which projects posteriorly from the precuneus and superior parietal lobule to the superior frontal gyrus and the anterior cingulate cortex (Schmahmann and Pandya, 2006, Schmahmann et al., 2008, Thiebaut de Schotten et al., 2011, Thiebaut de Schotten et al., 2012). Additionally, disrupted structural connectivity and rightward asymmetry of the SLF II was observed in the ASD group relative to the control group. The SLF II structurally connects the anterior parietal sulcus and the angular gyrus to the posterior superior and middle frontal gyri / dorsolateral prefrontal cortex (DLPFC) (Schmahmann and Pandya, 2006, Schmahmann et al., 2008, Thiebaut de Schotten et al., 2011, Thiebaut de Schotten et al., 2012). Connectivity between the anterior cingulate cortex, the DLPFC and the inferior parietal cortex are thought to facilitate cognitive control (Corbetta et al., 2008, Fan et al., 2012, Matsumoto and Tanaka, 2004). Interestingly, several studies have reported aberrant functional connectivity during cognitive control (Agam et al., 2010, Solomon et al., 2009), response inhibition (Kana et al., 2007) and response monitoring tasks in ASD (Thakkar et al., 2008) thus it is plausible that white matter anomalies in the SLF may contribute to disrupted functional connectivity observed in these studies. Furthermore, abnormal structure of the white matter in the SLF lends support to the

proposed hypothesis that ASD may be characterised by disrupted long-range connectivity (Muller et al., 2011, Kana et al., 2014b, Wass, 2011). The branches of the SLF have been associated with the dorsal and ventral attention networks (Thiebaut de Schotten et al., 2011) thus it is reasonable to suggest that stronger structural connectivity, reflected by greater FA and CL values, may support the increased functional connectivity shown during successful attention orienting in a subset of ASD participants.

7.2.5.3 Widespread Disrupted Structural Connectivity is Apparent in ASD

The results of the TBSS study were obtained from the largest ASD sample using this approach to date and have added to the growing body of literature supporting the theory of disrupted cortical connectivity in ASD [see (Vissers et al., 2012) for review]. In the current study, abnormal structural connectivity was characterised by widespread reduced FA in white matter. The findings support a central role for the corpus callosum (CC) in ASD pathology. Functional connectivity studies have shown that neural regions that are functionally connected via the CC are aberrant in ASD (Just et al., 2007, Kana et al., 2006, Mason et al., 2008). Additionally, reduced CC size was found to be associated with reduced functional connectivity during executive functioning (Just et al., 2007), theory of mind (Mason et al., 2008), sentence comprehension (Kana et al., 2006) and at rest (Cherkassky et al., 2006) thus a link between disrupted functional and structural connectivity is plausible. Reduced FA was observed in the anterior thalamic radiation (ATR) and similar associations have been postulated with regard to the ATR whereby both abnormal thalamo-cortical functional connectivity (Kleinhans et al., 2008, Mostofsky et al., 2009, Mizuno et al., 2006, Nair et al., 2013, Shih et al., 2011)

and disrupted white matter microstructure (Bakhtiari et al., 2012, Barnea-Goraly et al., 2010, Cheon et al., 2011, Shukla et al., 2011) have been reported in ASD. There is less support for the functional findings in other white matter tracts identified in the present study, e.g. the forceps major and minor, thus their potential role in ASD pathology is less understood. The forceps major and minor, cingulum and inferior longitudinal fasciculus have been associated with visual perception, executive functioning, emotional processing and face recognition (Fox et al., 2008) (Catani, 2006, Rudrauf et al., 2008, Tamura et al., 2007, Miller and D'Esposito, 2005) which are impaired in ASD thus disrupted white matter may provide a neurobiological target for these deficits in ASD.

7.2.5.4 ASD is Characterised by Aberrant Cortical Thickness, Surface Area and Cortical Folding Patterns

Results from the analysis of cortical structure are in keeping with neuropathological and neurobiological findings of atypical cortical development in a large ASD sample (Zikopoulos and Barbas, 2013, Redcay and Courchesne, 2005, Frith, 2003, Casanova et al., 2006). In ASD relative to controls, greater cortical thinning was observed in the left supramarginal, precentral and superior frontal gyri. In adolescence, cortical thinning is thought to occur, possibly due to synaptic elimination and dendritic arborisation (Sowell et al., 2004, Raznahan et al., 2011, Mills and Tamnes, 2014, Wierenga et al., 2014) thus increased cortical thinning in ASD may reflect excessive synaptic removal and dendritic pruning in the disorder. It may also be possible that aberrant cortical thinning in ASD may reflect a process that follows the same trajectory as typically developing individuals but occurs at a different rate.

Greater surface area was identified in the bilateral superior frontal, precentral and temporal gyri, left precuneus and the right lateral occipital region. The integrity of the surface area is considered driven by the number of minicolumns, neuronal structural subunits within the cortex (Rakic, 1988, Rakic, 1995). Post-mortem studies have observed a greater volume of minicolumns in ASD adult brains (Casanova et al., 2006, Buxhoeveden et al., 2006). Therefore, the current findings are in keeping with this finding of increased numbers of minicolumns in ASD. Moreover, these findings may reflect a disruption in cortical cytoarchitecture during development, which may subsequently lead to disrupted cortical connectivity (Casanova and Trippe, 2009). The findings of this study suggest that disrupted cortical formation occurs in ASD. Greater gyrification in the right inferior temporal region and reduced sulcal depth in the anterior cingulate and insula was observed in the ASD group relative to controls. Cellular studies have shown that increased cortical folding is associated with an abundance of basal radial glial cells (Borrell and Gotz, 2014). The Van Essen (1997) model of convolution development postulates that disrupted cortical folding reflects aberrant structural connectivity due to tension applied by radial glial cells during neuronal development. In light of this theory, the current findings of increased gyrification and reduced sulcal depth may reflect disrupted structural connectivity in ASD. Moreover, given the role of frontal regions in executive function (Hill, 2004), the aberrant thinning, surface area and gyrification identified in the frontal cortex in ASD may offer insight into the neurobiological abnormalities underpinning executive dysfunction. Collectively, these findings indicate that grey matter structural abnormalities in ASD can be characterised by distinct cortical metrics thus signifying the importance of investigating these measures independently.

7.2.5.5 Age-Related Differences in Grey and White Matter Structures in ASD

Age-related differences between the ASD and control groups were observed in gyrification of the right superior frontal region and in the white matter organisation of the left ATR. The disparity in gyrification was the result of an age-related decline in gyrification present in the control group but not the ASD group, possibly due to an excess of radial glial cells maintaining the cortical folding structure in ASD. This corroborates the finding of increased glial density in post-mortem DLPFC tissue (Morgan et al., 2010) and indicates that atypical neural development continues throughout the lifespan. On the other hand, the difference in white matter architecture in the ATR was due to an age-related increase in FA in the ASD group but not in controls. This observation may reflect a relative delay in white matter maturation over time. Additionally, previous studies have postulated that the findings may relate to the normalisation of white matter architecture over time (Kleinhans et al., 2012, Bakhtiari et al., 2012). Together, these findings suggest that the developmental trajectory of grey and white matter is divergent in ASD relative to controls however the consequences of this are not yet well understood.

7.2.5.6 Discrepancy in Findings of Disrupted White Matter Organisation Between Studies

It must be noted that the results identified in the CSD based tractography study of the three fronto-parietal branches of the SLF (Chapter 4) differed in comparison to the findings observed in the data-driven whole brain TBSS analysis of white matter organisation (Chapter 5). In the tractography study, increased FA was demonstrated in the left SLF I and the right SLF II however, in the TBSS study,

widespread reduced FA was observed including a posterior region of the right SLF. These analyses were performed in the same cohort so demographic or clinical effects cannot account for the discrepancies. It is likely that the different methodological approaches used influenced findings between these two studies. As previously described, the diffusion tensor model is unable to address regions of crossing or kissing fibers thus can influence FA values (see Chapter 4, Section 4.1.3). The reduced FA observed in the TBSS analysis in less complex neural regions such as the corpus callosum is likely a true representation of reduced structural organisation however the reduced FA identified in tracts passing through more complex neural regions such as the thalamic radiation, inferior and superior longitudinal fasciculus or the corona radiata may been impacted by the limitations of the TBSS method. It is probable that white matter abnormality in ASD is characterised by a pattern of both increased and reduced FA in distinct tracts at different time points throughout development. Therefore, while TBSS is beneficial for highlighting white matter structural abnormalities, it should be supplemented with more advanced methods such as CSD based tractography to further elucidate the nature of these findings. With respect to the divergent direction of FA in the SLF across the analysis methods, methodological limitations in addition to localisation of the region are liable to have attributed to the discrepancy. It has been suggested that tractography approaches are less robust and reliable at the extreme end of the tracts where the difference in the right SLF was observed in the TBSS analysis. This region also appears to correlate with the right SLF I branch which did not demonstrate a between-group difference in the CSD based tractography analysis thus the small difference observed may have been discounted by averaging of FA along the entire tract. These discrepant findings illustrate the crucial

need for replication of findings in order to make inferences about the anatomical pathology of ASD.

7.2.5.7 Multi-modal Imaging Methods Identify the Anterior Cingulate as a Central Region involved in ASD Pathophysiology

Interestingly, abnormalities relating to the anterior cingulate featured strongly across all studies using distinct MRI methods even though the study populations differed. In the functional connectivity analysis, stronger functional connectivity between the anterior cingulate and regions of the VAN appeared to play an important role in achieving typical behavioural performance. In the CSD based tractography analysis, disrupted structural connectivity was illustrated in the left SLF I which projects between the anterior cingulate cortex and parietal regions. Investigation of cortical metrics demonstrated that reduced sulcal depth was apparent in the ASD group relative to the control group in the anterior cingulate cortex while reduced white matter organisation was apparent in the anterior region of the cingulum in the TBSS analysis. Given the central role of the anterior cingulate in cognitive control, executive functioning, social cognition and decision making (Corbetta et al., 2008, Fan et al., 2012, Matsumoto and Tanaka, 2004, Ashwin et al., 2007, Shafritz et al., 2005) these findings provide neurobiological support for the anterior cingulate contributing to the behavioural manifestations of ASD.

7.3 Limitations and Considerations

7.3.1 Limitations of Sample Population

Participants with ASD in the current study were all high functioning (HFA) right-handed males assessed using the ADI-R and the ADOS-G; the instruments used to clinically diagnose ASD. Given that gender, age and IQ have been shown to affect cortical measures (Clayden et al., 2012, Wierenga et al., 2014), all ASD participants were gender, age and IQ matched with typically developing individuals to minimise confounding factors. While the sample population used in this study was extremely well matched between experimental groups, the ASD cohort of high functional males is not representative of all individuals with the disorder. Females and lower functioning individuals (LFA) with ASD were not included in the current study. It has been suggested that at least 1 in 4 individuals with an ASD diagnosis are female and at least 45% have an intellectual disability [see (Lai et al., 2014) for review]. One of the few studies that have compared the overall whole brain neuroanatomical structure of HFA and LFA participants identified differences between the groups in cortical folding patterns and sulcal depth (Nordahl et al., 2007) demonstrating the importance of future research incorporating these groups to delineate neural abnormalities in the disorder.

7.3.2 Considerations Regarding the Influence of the Developmental Trajectory on Brain Structure

The divergence in developmental trajectory shown in select brain regions in grey and white matter whole brain analyses highlights the importance of considering age,

or the developmental trajectory when interpreting results. The current study included individuals who ranged in age from 10 – 25 with the mean age in mid-adolescence therefore results should be compared with results of studies using a similar age group. Studies often employ linear regression analyses to investigate age related difference between experimental groups however evidence suggests that development of neural tissue is not linear nor is it uniform across brain regions or cortical measures (Mills and Tamnes, 2014, Giedd et al., 2014). Subsequently, region specific non-linear statistical models are required to investigate the development of grey and white matter in ASD. These cross-sectional studies are limited as inferences are based on developmental trends across individuals therefore longitudinal studies at several time points may be more beneficial. In neurotypical adolescents, longitudinal studies of cortical structure have strived to identify patterns of grey and white matter development (Giedd et al., 2014, Lebel and Beaulieu, 2011). The few longitudinal studies that have investigated neural substrates in ASD have been hampered by relatively small sample sizes thus lacking statistical power (Hua et al., 2013) and lack of matched intellectual ability (Hardan et al., 2009, Zielinski et al., 2014). Large longitudinal studies of well matched ASD and neurotypical cohorts would enhance the understanding of neuro-developmental trajectory changes and could help determine if early aberrant trajectories can predict the onset of ASD (Zielinski et al., 2014).

7.3.3 Difficulty Attributing Clinical Relevance to Neuroimaging Findings

To date, given the heterogeneity of the disorder, it has proven difficult to attribute neuroanatomical abnormalities to clinical impairments, which is key to using

neuroimaging methods to aid early diagnosis of ASD. Many studies have used sub-domain scores or total scores calculated from the ADI-R and/or the ADOS to assess the relationship between clinical features and neuroanatomy, however these assessments are used to establish ASD diagnosis rather than to measure symptom severity, thus are not normally distributed. To combat this issue, the current study implemented two metrics; social and communication deficits (SCD) and restricted repetitive behaviours (RRB) derived from the ADI-R using a mixed-factor model (Georgiades et al., 2013) because these are shown to be heritable (Liu et al., 2011a) and therefore likely to be more biologically valid. However confounds persist notwithstanding this approach. The ADI-R item level scores reflect childhood behaviour and while they may reflect a trait that is heritable and therefore reflected in abnormal structure or function, they may not be conducive for evaluating the association between current clinical presentation and neural abnormalities. Furthermore, as with most neuroimaging studies, the use of clinical scales such as the ADI-R and ADOS presents statistical barriers due to correction for multiple comparisons thus behavioural findings in the current study are presented as exploratory. Implementation of high-resolution MRI approaches such as CSD tractography in larger sample sizes characterised by clinical symptomology may improve understanding of clinical features and their relationship to neural structure.

7.3.4 General MRI Limitations

Some general limitations of MRI methods are evident in the literature. Changes in neural activation can be influenced by a number of external causes such as motion artefacts, position of the subjects' head in the B0 field and head coil, image noise, stability of the magnetic field, inhomogeneities of the field, shimming differences, level

of arousal and random cognitive process during the scanning session (Loubinoux et al., 2001). In the current study, cardiovascular noise or movement due to respiratory activity, which can interfere with the neural BOLD signal (Birn et al., 2006) was not controlled for during data acquisition. It may be beneficial to control for these sources of neuronal noise to increase confidence in data acquisition in future studies. The current study used advanced methodologies including optimal preprocessing methods to address any additional confounding factors that are not yet widely controlled for in ASD research.

7.3.5 Limitations and Considerations Regarding Attention Orienting Functional Connectivity Analysis

Given that a section of this thesis was dedicated to evaluating attention orienting in ASD, it may have been beneficial to have an external measure of attention such as eye tracking. Eye-tracking can record focused gaze and arousal during task performance (Bradley et al., 2008) thus it could be determined if slower performance on the spatial attention orienting paradigm could be explained by a reduced focus or interest in the task rather than an inability to orient attention as rapidly as neurotypical participants during invalid trials. Although there was no between-group difference in attention orienting performance in the current study, it may be best practice to implement a measure of finger-tapping speed to determine if motor control may be a confound when interpreting results (Liu et al., 2006). These tests monitor response time (RT) and use RT as a measure of motor coordination.

7.3.6 Lack of Clarity Regarding the Contribution of Greater Connectivity to ASD Pathology

Research indicates that neural regions operate in a series of neural networks in order to sub-serve higher cognitive functioning thus it holds that the impaired cognitive features in ASD may be attributed to compromised brain networks. This theory of disrupted cortical connectivity in ASD has largely been the focus of neuroimaging research over the past decade but many questions remain unanswered. In the current study, greater functional and structural connectivity was demonstrated between fronto-parietal regions however the meaning of greater connectivity in terms of ASD pathology is not clear from the existing literature. Some research suggests that increased functional connectivity reflects a compensatory mechanism engaged by individuals with ASD to achieve neurotypical behaviour. On the other hand, some reports suggest that increased functional connectivity may reflect a hyperspecialisation rather than more efficient connectivity thus is detrimental (Maximo et al., 2014). Increased functional connectivity was demonstrated during a cognitive control task in which greater error rates were observed in ASD (Solomon et al., 2013). Additionally, increased functional connectivity was associated with greater restricted repetitive behaviours (Agam et al., 2010, Monk et al., 2009) and greater social and communication deficits (Weng et al., 2010, Supekar et al., 2013)

Additionally, the relationship between greater structural connectivity and ASD symptomology remains unclear. Increased FA is thought to reflect greater myelination, axonal density and fiber organisation (Beaulieu, 2002). As greater FA increases during typical development in conjunction with the development of higher-order cognitive

processes, researchers have postulated that increased FA facilitates the development of complex cognitive abilities (Liston et al., 2006, Luna et al., 2004, Geier and Luna, 2009, Ashtari et al., 2007). Thus it is possible that increased structural connectivity in ASD may represent an enhanced cognitive processing ability relative to controls. Conversely, greater structural connectivity may reflect an over-complexity of white matter organisation resulting in the inability to integrate neural signals thus reducing cognitive ability i.e. it could increase the signal to noise ratio.

It is possible that the impact of greater functional connectivity and structural organisation on behavioural presentation may depend on cognitive demands. A few studies have examined the link between functional and structural connectivity in the same ASD cohort (Delmonte et al., 2013, Kana et al., 2012, McGrath et al., 2013b, Mueller et al., 2013, Nair et al., 2013) but the findings have been inconsistent. Future studies focused on elucidating the link between the structural and functional connectivity in specific ASD phenotypes is crucial to understanding the involvement of connectivity abnormalities in ASD pathology.

7.4 Future Directions

7.4.1 Use of Multi-Modal Approaches Required in ASD

Multiple neuroimaging modalities were used to investigate cortical structure and connectivity differences in ASD in the current study and while a sub-sample of the participants were involved in each study; the same sample was not used across analyses. Using several MRI modalities within a large homogenous sample would allow a more in depth analysis of the links between brain functional and structural

connectivity and enable researchers to identify patterns of abnormality. This type of data would increase the level of understanding of the pathophysiology of atypical connectivity in ASD and may offer neuroanatomical biomarkers which could be used for the biological classification of ASD (Ecker et al., 2010). As causation cannot be extrapolated from DTI or fMRI findings alone, implementing MRI approaches in conjunction with other imaging methods such as magnetic resonance imaging spectroscopy (MRS) and electroencephalography (EEG) can help elucidate the pathophysiology of ASD. MR spectroscopy non-invasively measures neural metabolites concentration. It has been suggested that there an excitatory/inhibitory imbalance relating to GABA/Glutamate production present in ASD which may been reflected by disrupted cortical connectivity (Belmonte et al., 2004, Rubenstein and Merzenich, 2003). Thus, combining MRS and MRI modalities may offer novel insights into the cellular mechanisms driving under- and overconnectivity in ASD.

Use of MRI alone can limit the research of certain ASD populations. From a practical viewpoint, inclusion of LFA participants in neuroimaging research is challenging. Due to the reduced cognitive functioning of LFA individuals, explaining scanning procedures is difficult. Furthermore, given their behavioural difficulties, staying in the scanner for time required for data acquisition is improbable. Excess motion in the scanner in subjects with an intellectual disability is common (Power et al., 2012) thus, as MRI modalities are sensitive to subject motion (Chang et al., 2005, Jones and Cercignani, 2010, Haller and Bartsch, 2009, Fox and Raichle, 2007), the data obtained from such scans is likely to be unworkable. Therefore combining modalities is not also important for establishing potential neural biomarkers but it is also crucial for incorporating subject populations not suitable for MRI studies. For example,

determining how MRI based connectivity is related to more acceptable and less restrictive modalities such as EEG would enable researchers to make inferences about brain connectivity in LFA from EEG data alone.

7.4.2 Significant Role for Genetic Imaging in ASD

The neurobiology of ASD is complex and considerable heterogeneity exists among individuals. Currently, ASD cohorts included in research studies are defined by behavioural observations based on measures such as the ADI and the ADOS irrespective of co-morbidities or language difficulties which are likely to influence results [see (Lenroot and Yeung, 2013) for review]. Addressing the heterogeneity in ASD symptomology is an important challenge for future research. Thus characterisation of the neurobiological anomalies that underpin ASD and development of novel therapies for ASD requires input from multiple disciplines.

Genetic studies have demonstrated success in identifying genomic risk factors for ASD [see (Won et al., 2013) for review]. Researchers have indicated that study samples that are not differentiated by genotype may obscure important biological information pertaining to the disorder. Use of endophenotyping may elucidate pathways of the disorder through the effects of risk variants on neuroanatomy (Meyer-Lindenberg, 2010). Additionally, imaging-genetic studies may provide novel insights into the clinical features of ASD [see (Ameis and Szatmari, 2012) for review]. Given that genes involved in synaptogenesis, synaptic function and neuronal structure have been implicated in ASD pathology (Marshall and Scherer, 2012, State and Levitt, 2011) and neuroimaging studies have demonstrated aberrant structure, function and connectivity [see (Vissers et al., 2012, Anagnostou and Taylor, 2011, Pina-Camacho et al., 2012) for reviews],

merging the two methods may bridge the gap in current knowledge regarding atypical neural development in ASD and provide novel targets for therapeutic interventions.

7.4.3 Data Sharing Initiatives are Crucial for Developing the Field of ASD Research

A major challenge for neuroimaging research, not only in ASD but the majority of neuropsychiatric disorders is to deliver clinically relevant insights to inform early diagnosis and therapeutic interventions (Malhi and Lagopoulos, 2008). Establishing viable neuroimaging biomarkers will require large sample sizes in addition to consistent diagnostic, data acquisition, scanning and preprocessing protocols. Data sharing can also uncover errors, increase statistical power and improve data quality (Poline et al., 2012). Progress towards sharing of neuroimaging data has been slow, possibly due to factors such as heterogeneous data acquisitions, study populations and ethical restrictions such as participant confidentiality. Two prominent data-sharing consortiums have been established which have incorporated ASD, the Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) http://enigma.ini.usc.edu/ and the Autism Brain Imaging Data Exchange (ABIDE) (http://fcon_1000.projects.nitrc.org/indi/abide/). The ENIGMA consortium has collected genetic and structural imaging data from over 12,000 individuals with a variety of disorders including ADHD, schizophrenia, addiction and depression as well as typically developing individuals and it has just introduced an ASD imaging working group. The ENIGMA groups' first mandate was a genome-wide association study identifying common variants in the genome associated with hippocampal or intracranial volume (Thompson et al., 2014). ABIDE has aggregated resting-state data

from 539 ASD and 573 controls from several sites worldwide. Resting-state fMRI was selected for collection by this consortium due to its robust test-retest reliability and lack of obstacles in collating the data i.e. no task is involved (Di Martino et al., 2013). Furthermore, resting-state fMRI data can be 'fruitfully aggregated' for discovery and replication (Di Martino et al., 2013). Methodological implications must be considered prior to sharing neuroimaging ASD data. Both the ABIDE and ENIGMA consortiums collected the MRI data retrospectively therefore diagnostic, scanning and data acquisition protocols are not uniform and may influence findings. Standardised processing pipelines have been implemented in both these data-sharing consortiums in order to compensate for these potential confounds. Future data-sharing initiatives must strive to standardised protocols prior to data collection across sites to further eliminate any discrepancies in data collection. Another issue for data sharing is insufficient reliability of structural imaging methods across scanners (Kruggel et al., 2010). The use of the MRI scanners with a higher field strength is likely to improve reliability and replication given the production of better quality data from scanners with a greater field strength (Linden, 2012). With the inclusion of additional phenotypic, demographic and genetic data, neuroimaging data sharing consortiums have the potential to substantially improve understanding of ASD neuropathology.

7.4.4 Continuation of Current Work

7.4.4.1 Future Functional Connectivity Analyses

The functional connectivity analyses performed in Chapter 3 will be expanded upon by performing a dynamic functional connectivity analysis. The functional connectivity analyses performed in the current study assumes that functional networks

are static however dynamic functional connectivity analyses can probe the changes in functional connectivity of the dorsal and ventral attention networks that occur over time. This type of analysis can elucidate how these networks interact throughout performance of the attention orienting task (Sakoglu et al., 2010). Furthermore, the PPI analysis approach used cannot determine causal relationships between brain regions. Effective connectivity methods such as dynamic causal modelling (DCM) can be employed to explain observed dependencies. DCM involves producing a neurobiologically plausible neuronal model of interacting cortical regions supplemented by a biophysically plausible forward model of how synaptic of neuronal activity can be transformed into a measured signal thus allowing the parameters of the neuronal model to be estimated from observed data (Friston et al., 2003). Implementation of DCM can determine which neural region, in response to attention orienting task demands, is driving changes in functional connectivity.

7.4.4.2 Future Structural Connectivity Analyses

As previously described in Chapter 4, Section 4.1.3, CSD based tractography is a new method that was developed to overcome flaws in previous methodologies used to investigate white matter organisation. This method has only been used in one prior study examining disrupted white matter in ASD (McGrath et al., 2013a). It is clear from the current TBSS study that anomalies are present in several white matter tracts in ASD relative to controls. Using CSD methods, these tracts can be extracted and whole tract as well as tract segmentation analyses can be performed. These in-depth analyses of white matter microstructure can provide more clarity regarding the neurobiological underpinnings of ASD.

7.4.4.3 Multi-Modal Approaches to Investigate and Corroborate Current Findings

Given the importance of combining methods in understanding ASD pathology, following on from this thesis, analyses will involve directly linking functional MRI, diffusion MRI and structural MRI data. Functional connectivity MRI data during attention orienting with the 'McGrath' cohort (see Chapter 2, Section 2.2) will directly linked with diffusion MRI using the approach described in McGrath et al (McGrath et al., 2013b). For this analysis, ROIs can be obtained based on regions of significant differences between groups in one modality i.e. regions which illustrated significant between group differences in functional connectivity which can then be converted into diffusion MRI space for analysis of potential underlying structural connectivity differences between these regions. Elucidating the relationship between the structural and functional connectivity is vital for understanding the contribution of connectivity abnormalities to ASD. Furthermore, diffusion MRI data will be directly linked with structural MRI in 45 ASD and 45 age and IQ matched controls to determine if there is is a concordance between grey and white matter abnormalities in ASD. It has been hypothesised that correlation of cortical thickness values between two different regions may reflect the underlying coherence of structural connectivity (Cauda et al., 2014) thus combining diffusion and structural data in the same cohort permits investigation of this in ASD in addition to providing further consolidation and localisation of anatomical abnormalities in ASD.

Resting state data was also collected from the 'Fitzgerald' population. Considering that functional connectivity is thought to be state-dependent (You et al., 2013), an investigation of how functional connectivity at rest compares with functional

connectivity during a cognitive flexibility task also performed in the 'Fitzgerald' cohort (see 7.4.4.4) will be performed between groups. This may guide our understanding of how increased and/or decreased functional connectivity may underpin ASD pathology. Similarly, resting state MRI will be combined with diffusion MRI data from this sub-sample to directly investigate if disrupted resting state neural networks are underpinned by abnormal white matter organisation.

Finally, an endophenotypic approach is crucial to uncovering the mechanisms involved in the development of ASD. Genetic data has been collected from a sub-sample of participants involved in the grey matter cortical structure analyses (Chapter 6). It is hoped that genetic information can be obtained from the remaining individuals in addition to the recruitment of further individuals with ASD for genetic-imaging analyses. Ideally, it would be beneficial to collect neuroimaging and genetic data not only from individuals with ASD but also their siblings and biological parents to build a comprehensive picture of the neurobiological underpinnings of ASD in order to inform targeted interventions and provide novel biomarkers for early diagnosis.

7.4.4.4 Study of Executive Dysfunction Theory in Light of the Abnormal Connectivity Theory – A Focus on Cognitive Flexibility

The finding of disrupted functional connectivity during attention orienting is consistent with both the executive dysfunction theory of ASD and the aberrant cortical connectivity theory of ASD. However, executive function is an umbrella term for a wide range of cognitive processes such as response inhibition, cognitive flexibility, impulse control and working memory, some of which have yet to be explicitly studied in respect of the abnormal cortical connectivity theory. There is a striking lack of research

regarding cognitive flexibility in ASD. Cognitive flexibility, also referred to as set-shifting, is the ability to switch or shift to a different thought or action according to changes in situation (Sanders et al., 2008) and is considered an essential component of higher cognitive functions such as decision-making, problem-solving, reward processing and emotion regulation (Kim et al., 2012). Deficits in cognitive flexibility have been reported in ASD (Geurts et al., 2004, Ozonoff et al., 2004, South et al., 2007, Yerys et al., 2009) and may be associated with the core restrictive, repetitive behaviours characteristic of the disorder (Lopez et al., 2005, South et al., 2007). Cognitive flexibility appears to activate a network of regions including the ventrolateral-prefrontal cortex, anterior cingulate, parietal lobules, striatum and insula (D'Cruz et al., 2011, Monchi et al., 2001, Shafritz et al., 2005). Few neuroimaging studies have investigated this cognitive process in ASD. One study reported an increase in parietal activation (Schmitz et al., 2006) while another revealed reduced frontal, striatal and parietal activation (Shafritz et al., 2008). Taylor et al. (2012) reported age related changes in the insula and the ventro-lateral frontal regions. A functional connectivity study of cognitive flexibility offers a novel avenue from which to pursue the disrupted executive function and cortical connectivity theories of ASD.

The 'Fitzgerald' cohort (see Chapter 2, Section 2.2) performed a cognitive flexibility task. This task was based on a paradigm previously used to investigate cognitive flexibility in individuals with obsessive-compulsive disorder (Figure 7.1) (Gu et al., 2008).

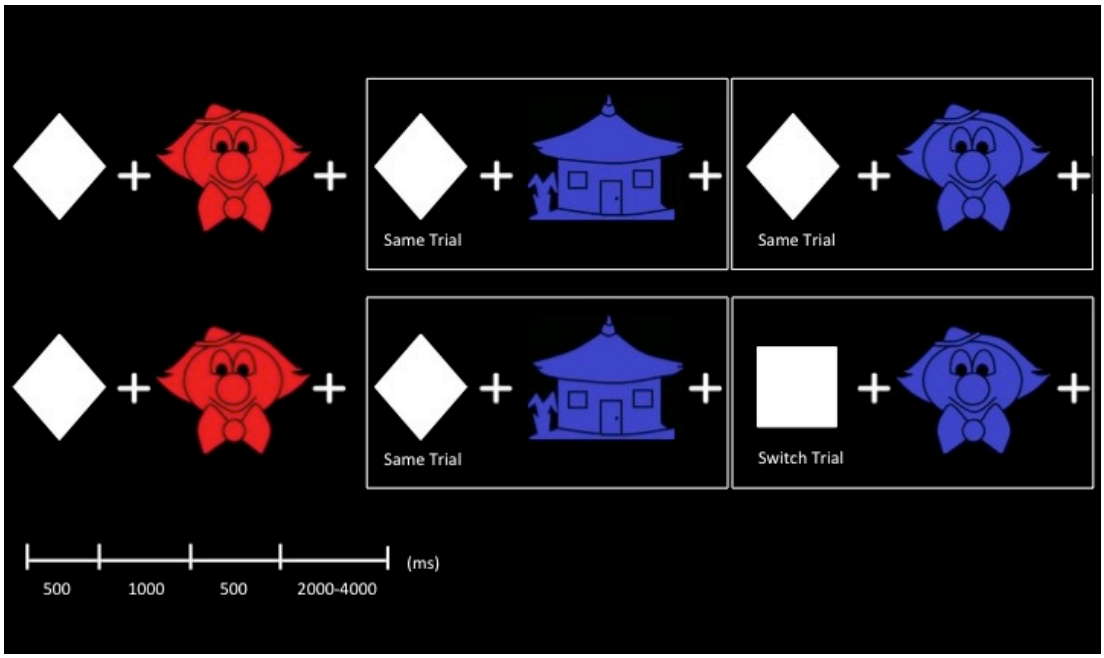

Figure 7.1 Example of Trial Types from Cognitive Flexibility Paradigm

A white cross was presented in the centre of the screen which turned green to coincide with the first pulse of the scanner which marks the beginning of the task. Two types of cue were presented, a diamond or a square. The diamond cued participants to respond to the colour of the subsequent picture (red or blue) and a square cued participants to respond to the shape of the subsequent picture (house or face). Each cue was presented for 500ms followed by an interstimulus interval of 1000ms. Each picture was presented for 500ms and the intertrial interval was jittered between 2000 and 4000ms. A total of 121 trials consisting of 30 switch trials and 90 same trials were presented. The first trial was discarded and all trials were presented in random order with interval between switch trials at least 16000ms.

To date, behavioural analyses have been performed but analysis of neuroimaging data have not yet completed. Behavioural results indicate that individuals with ASD made significantly more errors on switch trials than their control

counterparts. A functional connectivity analysis of this data will be performed in order to investigate if cortical connectivity is disrupted in individuals with ASD relative to controls during performance of set-shifting between trial types.

7.4.5 Contribution of Neuroimaging to ASD Research

The advent of neuroimaging methodologies in ASD research has undoubtedly advanced our understanding of the neurobiological underpinnings of the disorder. It established one of the most consistent findings on ASD research of early brain overgrowth reflected by greater volume (Carper and Courchesne, 2005, Aylward et al., 2002, Courchesne, 2004). ASD research can only benefit further from developments in the field of MR imaging. For example, the high-resolution data acquired from a 7T scanner can discriminate between the layers of the neocortex thus can provide novel information regarding its organisation. Prior to this, valuable information such as this could only be obtained from histological studies. This has implications for elucidating theories such as the minicolumn pathology theory and its relationship to disrupted connectivity in ASD.

Not only do imaging methods have the potential to provide biological classification of ASD, neuroimaging approaches also have the capacity to evaluate therapeutic interventions. Intensive remedial instruction in reading increased the white matter microstructure in children with impaired reading ability. Recent studies have indicated that behavioural interventions can result in normalisation of brain function (Voos et al., 2012) and can improve measures of white matter microstructure in the uncinate fasciculus in ASD (Matteo Pardini et al., 2012). It is apparent that

neuroimaging methods in combination with other modalities must be used to establish an understanding of the biology of ASD.

7.5 Final Conclusions

The overall findings of the studies presented offer support for the altered cortical connectivity theory of ASD. Functional connectivity of dorsal and the ventral networks was shown to be disrupted in ASD during attention orienting. Additionally, using two analytic approaches, results indicated that aberrant structural connectivity is present in ASD. It was also demonstrated that distinct cortical measures of grey matter structure are abnormal in ASD relative to neurotypical individuals. These findings have important implications for ASD research. Disrupted connectivity was characterised by both greater and reduced functional and structural connectivity. It indicates that both under- and over-connectivity contribute to ASD pathology. Identification of anomalies in distinct cortical measures of grey matter suggests that aberrant cortical development may be driven by multiple independent mechanisms. Cortical measures may have the potential to biologically deconstruct the heterogeneity of ASD and thus, in conjunction with genetic information, may offer novel targets for establishing neurobiological biomarkers for ASD diagnosis. The development of collaboration and data-sharing initiatives, implementation of new analytical approaches such as CSD based tractography in addition to the use of multimodal approaches is crucial to enhancing our understanding of how genetic factors and neural abnormalities confer risk for ASD. With a greater understanding of ASD pathology, novel therapeutic interventions and biologically informed diagnoses may be possible.

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Appendix A: Initial Contact Form

Parent Name: _____

Child Name: _____

DOB: _____

Handedness: _____

Contact Number: _____

Address: _____

_____

Medication: _____

Other medical issues / diagnoses: _____

_____

History of head trauma / Loss of consciousness: _____

Operation / Surgery: _____

Metal (Braces / Surgery – when / what?): _____

Claustrophobia: _____

Previous cognitive assessments (ADOS/ADI/IQ/NEPS) : _____

Age of Diagnosis (and by whom): _____

Additional Information: _____

_____

_____

Appendix B: Two-Factor Structure of the ADI-R Algorithm Items taken from Georgiades et al. (2013)

	No.	ADI-R diagnostic algorithm items (item no.)	SCD factor	FIRB factor
SCD	1	Direct gaze (item 50)	.405	.238
	2	Social smiling (item 51)	.509	.147
	3	Range of social expressions used to communicate (item 57)	.535	.131
	4	Interest in children (item 62)	.650	.020
	5	Response to approaches of other children (item 63)	.617	.031
	6	Showing and directing attention (item 52)	.656	.147
	7	Offering to share (item 53)	.546	.001
	8	Seeking to share enjoyment with others (item 54)	.608	.140
	9	Use of other's body to communicate (item 31)	.283	.145
	10	Offering comfort (item 55)	.607	.133
	11	Quality of social overtures (item 56)	.636	.100
	12	Inappropriate facial expressions (item 58)[a]	.063	.536
	13	Appropriateness of social responses (item 59)	.616	.025
	14	Pointing to express interest (item 42)	.626	.009
	15	Nodding (item 43)	.507	.097
	16	Head shaking (item 44)	.547	−.047
	17	Conventional/instrumental gestures (item 45)	.647	.045
	18	Spontaneous imitation of actions (item 47)	.583	.179
	19	Imaginative play (item 48)	.521	.199
	20	Imitative social play (item 61)	.507	−.006
FIRB	21	Unusual preoccupations (item 67)	.047	.414
	22	Compulsions/rituals (item 70)	.018	.348
	23	Hand and finger mannerisms (item 77)	−.007	.547
	24	Other complex mannerisms or stereotyped body movements (item 78)	.088	.595
	25	Repetitive use of objects or interest in parts of objects (item 69)	.203	.529
	26	Unusual sensory interests (item 71)	.100	.619

SCD = Social and Communication Deficits, FIRB = Fixed Interests and Repetitive Behaviours. SCD / FIRB factor columns denote how an item loads onto these factors.

Appendix C: Correlations between Behavioural Measures (SCD and RRB) and MRI Data

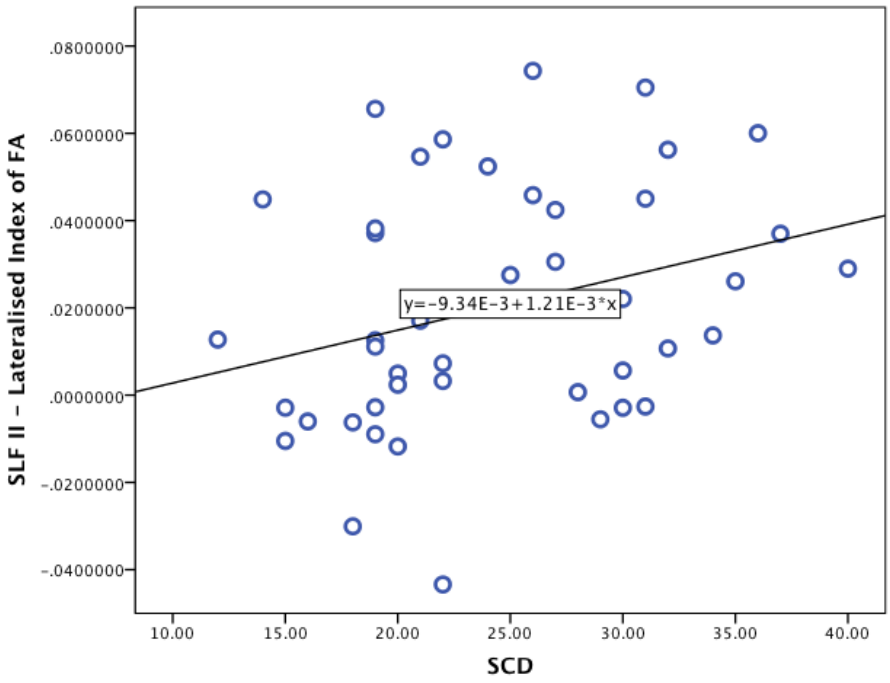


Figure 1. Correlation between Social and Communication Deficits and Lateralisation of Fractional Anisotropy in the Superior Longitudinal Fasciculus

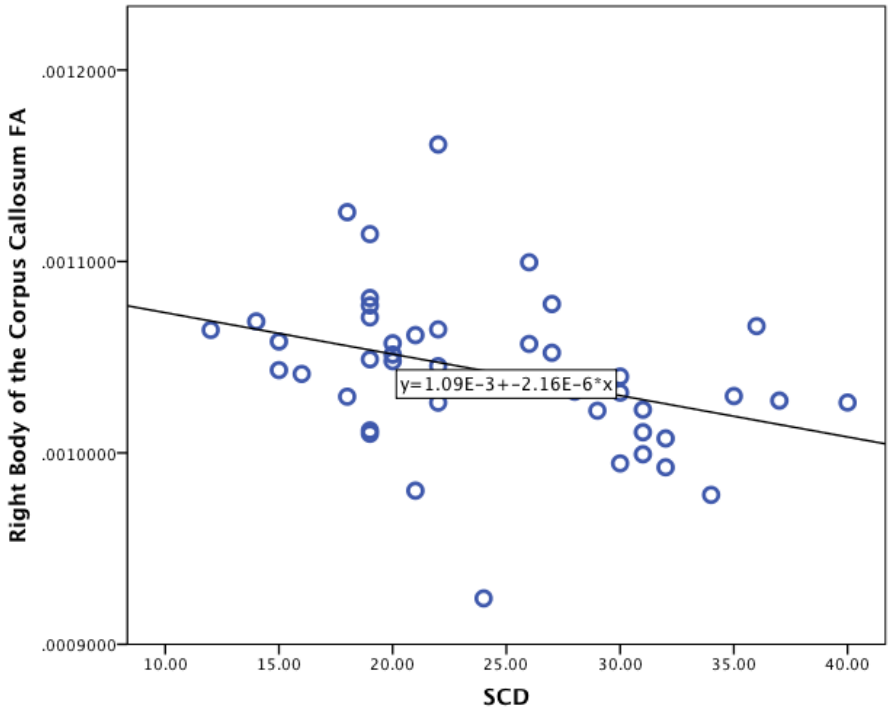


Figure 2. Correlation between Social and Communication Deficits and Fractional Anisotropy in the Right Body of the Corpus Callosum

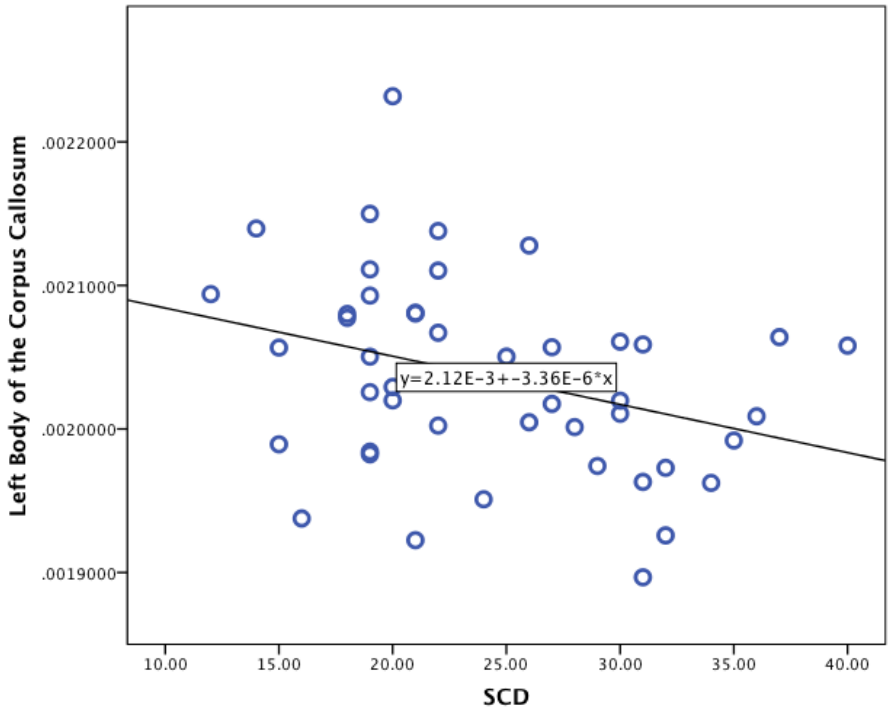


Figure 3. Correlation between Social and Communication Deficits and Fractional Anisotropy in the Left Body of the Corpus Callosum

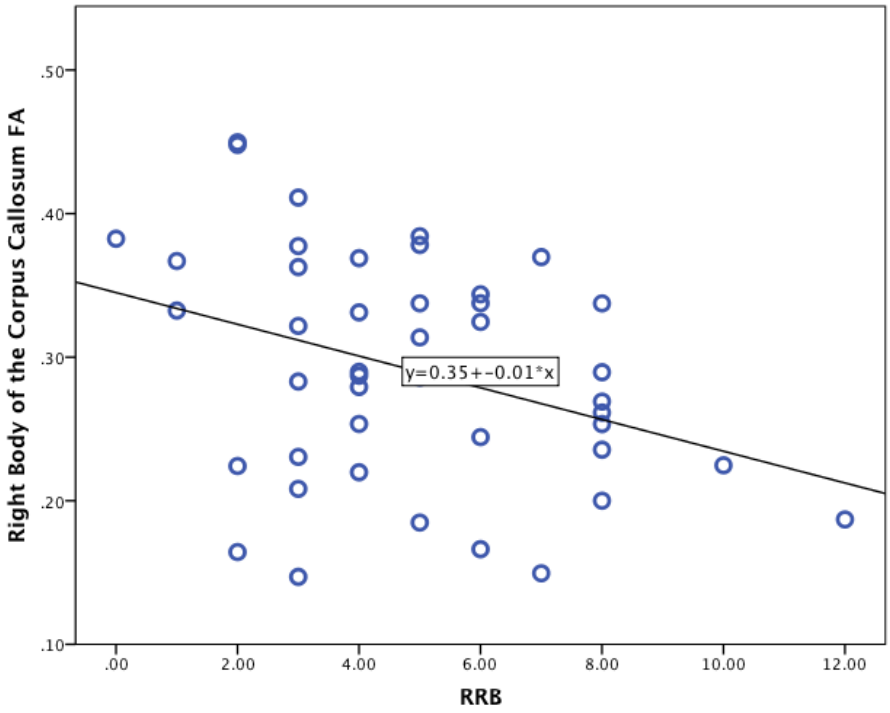


Figure 4. Correlation between Restricted and Repetitive Behaviours and Fractional Anisotropy in the Right Body of the Corpus Callosum

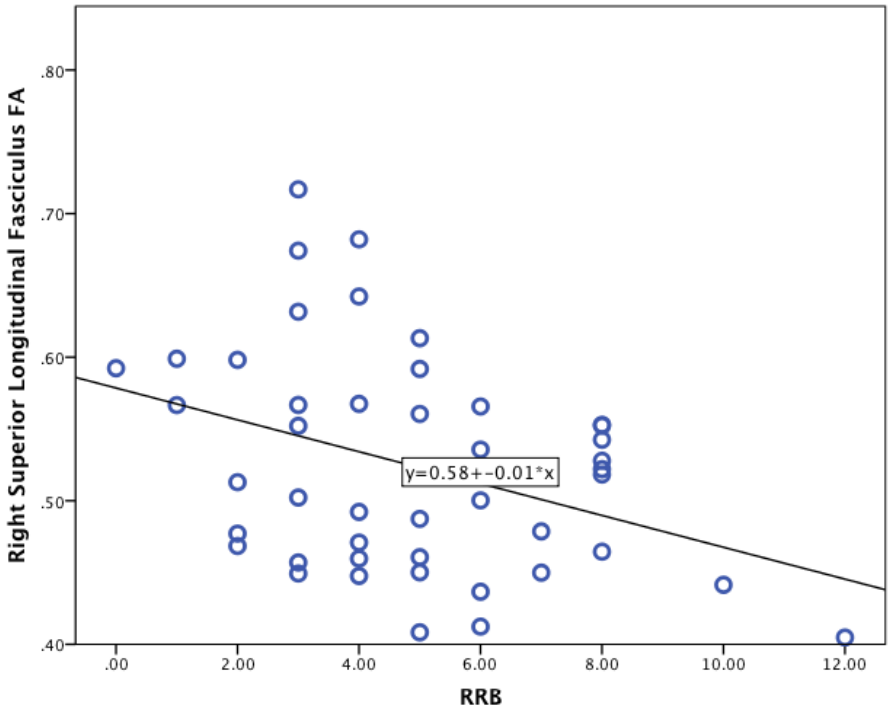


Figure 5. Correlation between Restricted and Repetitive Behaviours and Fractional Anisotropy in the Right Superior Longitudinal Fasciculus

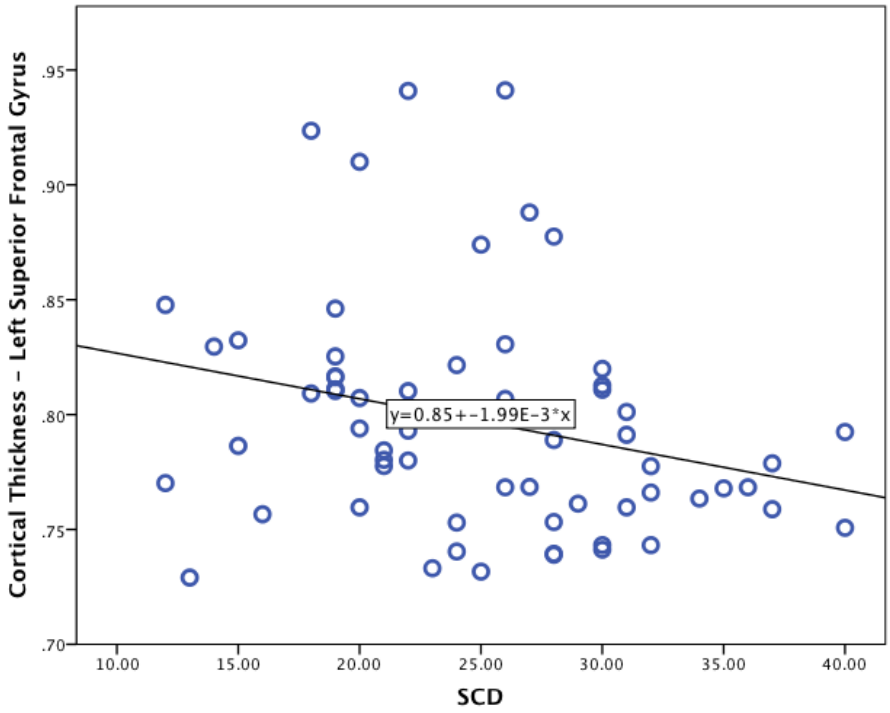
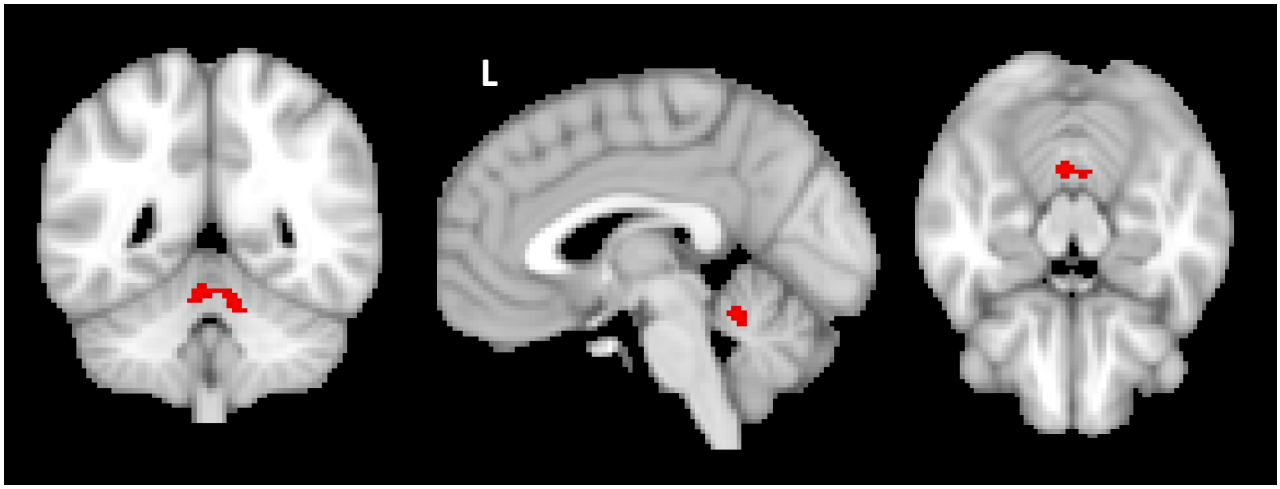


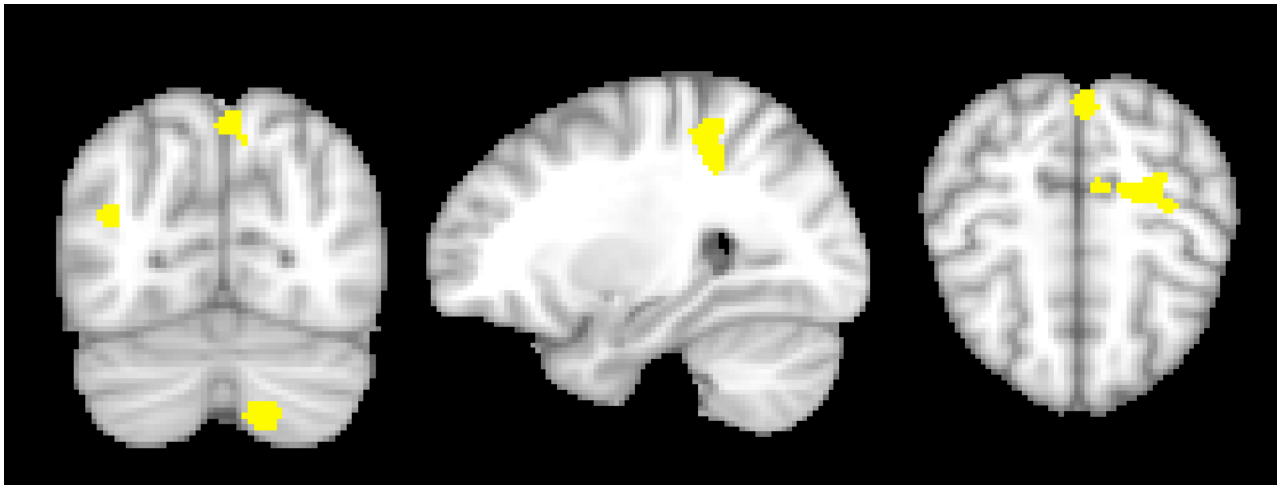
Figure 6. Correlation between Social and Communication Deficits and Cortical Thickness of the Left Superior Frontal Gyrus

Appendix D: Between-group Functional MRI Results of Attention Orienting Task

Cue-only trials: The ASD group showed a significant decrease in activation in the left and right precuneus in comparison to controls during cue-only trials. There were no other significant differences between groups during the cue-only condition of the task.



Invalid trials: The left cerebellum is the only region in which there was a group difference, where the ASD showed greater activation than the control group.



Valid trials: The control group showed greater activation in the right precuneus, left middle temporal gyrus and the right cerebellum in comparison to controls. The ASD group showed no regions of greater activation

Table 1. Between-group differences during cue-only, invalid and valid trials

	Region	Cluster Size (Voxels)	X	Y	Z	T-value	Direction
	Cue-only Trials						
L	Precuneus	261	12	64	22	3.82	C > ASD
R	Precuneus	Same Cluster	-8	76	26	3.63	C > ASD
	Invalid Trials						
L	Cerebellum	120	4	50	-18	3.91	C < ASD
	Valid Trials						
R	Precuneus	336	-26	42	44	5.08	C > ASD
L	Middle Temporal Gyrus	173	42	80	22	4.19	C > ASD
R	Cerebellum	115	-14	70	-48	3.66	C > ASD

Direction of between-group difference denoted with arrows, > = greater than, < = less than, R = Right, L = Left, C = Control group, ASD = Autism Spectrum Disorder group. All corrected for multiple comparisons, $p < 0.05$