Function and Structure of the Mirror Neuron System in Autism

by

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Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Deakin University

15.01.2014
I am the author of the thesis entitled: “function and structure of the mirror neuron system in autism”

Submitted for the degree of Doctor of Philosophy

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Publications

Chapter 2

Chapter 3

Chapter 5
Conferences

Chapter 2

Chapter 3

Abstract

The mirror neuron (MN) hypothesis of autism was assessed with three neuroimaging techniques: a blood oxygen level dependent (BOLD) contrast during a video task, functional connectivity (FC) and diffusion tensor imaging (DTI). A sample of 12 individuals with high functioning autism or Asperger syndrome (HFA/AS), and 12 typically developing (TD) individuals participated in the research. Brain regions associated with MN activity (pars opercularis, premotor cortex, inferior parietal lobule, superior temporal sulcus) were specified a priori, and then contrasted between the groups.

Study 1 tested for differences in participants’ BOLD response in MN regions whilst participants viewed hand gestures, and revealed that the HFA/AS group exhibited increased BOLD in the right premotor cortex and anterior cingulate, and reduced BOLD in temporal and occipital areas.

Thus, in Study 2 the premotor cortex and anterior cingulate were used as seed points to assess FC, a measure of temporal correlation in BOLD response from the chosen seed with the rest of the brain. Although no significant differences in FC between the groups were observed when seeding the anterior cingulate, the premotor cortex exhibited increased FC with the inferior parietal lobule and superior occipital gyrus in the HFA/AS group, and reduced FC with the insula and cuneus.

Based upon these differences in brain function, Study 3 utilized DTI to examine white matter fasciculi believed to be associated with the MN system (superior longitudinal fasciculus and cingulum bundle). The HFA/AS group
demonstrated widespread white matter deficits across the brain compared to TD individuals that were more pronounced in the left hemisphere. This included the two hypothesized fasciculi.

Collectively, the data from this thesis supports the hypothesis of abnormal function and structure of the MN network in individuals with HFA/AS. Study 3 revealed white matter impairments in the MN network were situated in the inferior parietal lobule. It is theorized structural impairments in this region may contribute to the functional impairments identified in the premotor cortex in study 1 and 2. More broadly however, all three studies of this thesis demonstrate that participants with HFA/AS possess anomalies distributed across the whole brain. These deficits were more pronounced in the left hemisphere, which was attributable to anomalies in myelination. This suggests that autism may be linked to a number of generalized deficits in neural processing such as cortical inefficiency, distributed across a large number of networks across the brain. These deficits most likely escalate from a history of abnormal neurodevelopment.
# Table of Contents

1 Chapter 1: Autism

1.1 Introduction 2

1.2 Autism 3

1.2.1 History 3

1.2.2 Definition and Diagnosis 8

1.2.3 Asperger’s Syndrome and High Functioning Autism 15

1.2.4 Prevalence 17

1.2.5 Comorbidity 21

1.3 Triad of Autistic Symptoms 24

1.3.1 Social Deficits 25

1.3.2 Communication Deficits 30

1.3.3 Restricted and repetitive behaviours 35

1.3.4 Relationship between the triad of symptoms 41

1.3.5 Conclusion 42

1.4 Cognitive Theories of Autism 42

1.4.1 Theory of Mind Hypothesis 43

1.4.2 Systemizing Theories 47

1.4.3 Executive Dysfunction Theory 50

1.5 Conclusion 54

2 Chapter 2: Biology of Autism 56

2.1 Introduction 57

2.2 Genetic basis of Autism 58

2.2.1 Heritability 58

2.2.2 Chromosomal Anomalies 60

2.2.3 Gene Candidates 65

2.2.4 Teratogens and Vaccines 70

2.2.5 Epigenetic Contributions 75
3.2.2 Stimuli
   3.2.2.1 Hand-Object
   3.2.2.2 Hand-Mouth
   3.2.2.3 Hand-Communicative
   3.2.2.4 Hand-Directive

3.2.3 Stimulus Presentation

3.2.4 fMRI Acquisition and Pre-processing

3.2.5 fMRI Image Analysis
   3.2.5.1 Within Groups Analysis
   3.2.5.2 Between Groups Analysis

3.3 Results
   3.3.1 Within Group Analysis
   3.3.2 Between Group Analysis

3.4 Discussion
   3.4.1 Hypothesized Findings
   3.4.2 Non-Hypothesized Findings
   3.4.3 Conclusion

4 Chapter 4: Study (2)

Functional Connectivity of the mirror neuron system in participant with autism

4.1 Introduction

4.2 Method
   4.2.1 Participants
   4.2.2 Resting-State Protocol
   4.2.3 Regions of Interest Selection
   4.2.4 fMRI Acquisition and Pre-processing
   4.2.5 fMRI Image Analysis
      4.2.5.1 Within Group Analysis
      4.2.5.2 Between Group Analysis

4.3 Results
4.3.1 Within Group Analysis 153
   4.3.1.1 Premotor Cortex Seed 153
   4.3.1.2 Anterior Cingulate Cortex Seed 156
4.3.2 Between Group Analysis 157
   4.3.2.1 Premotor Cortex Seed 157
   4.3.2.2 Anterior Cingulate Cortex Seed 160

4.4 Discussion 160
   4.4.1 Right Premotor Cortex 161
   4.4.2 Right Anterior Cingulate Cortex 164
   4.4.3 Final Considerations 165

5 Chapter 5: Study (3)
   Structural Connectivity of the mirror neuron system in participant with autism 168
   5.1 Introduction 169
   5.2 Method 174
      5.2.1 Participants 174
      5.2.2 fMRI Acquisition and Pre-processing 175
      5.2.3 Region of Interest Tractography 175
      5.2.4 Tract Based Spatial Statistics 177
   5.3 Results 179
      5.3.1 Region of Interest Tractography 179
         5.3.1.1 Inter-Rater Reliability 179
         5.3.1.2 Between Groups Analysis 180
      5.3.2 Tract Based Spatial Statistics 180
         5.3.2.1 Fractional Anisotropy 180
         5.3.2.2 Other Measures 184
   5.4 Discussion 184

6 Chapter 6: General Discussion 190
   6.1 Overall Summary 191
   6.2 Relevance to Mirror Neuron Hypothesis of Autism 192
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2.1 Study 1</td>
<td>192</td>
</tr>
<tr>
<td>6.2.2 Study 2</td>
<td>195</td>
</tr>
<tr>
<td>6.2.3 Study 3</td>
<td>199</td>
</tr>
<tr>
<td>6.2.4 Overall Implications</td>
<td>200</td>
</tr>
<tr>
<td>6.3 Alternative Explanations</td>
<td>203</td>
</tr>
<tr>
<td>6.4 Thesis Limitations and Future Research</td>
<td>207</td>
</tr>
<tr>
<td>6.5 Conclusion</td>
<td>208</td>
</tr>
</tbody>
</table>

References 210

Appendices 284
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Degree of handicap experienced by subtype of autism</td>
<td>8</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Rising prevalence of autism from 1977 to 1994</td>
<td>18</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Scatterplot depicting estimate rise in autism prevalence by sample size</td>
<td>20</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Point of eye fixation of an autistic and typically developing person during conversation</td>
<td>27</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Examples of stimuli in which autistic subjects failed to use gestalt principles</td>
<td>38</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Example of the Sally-Anne false belief task</td>
<td>44</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Example of the social attributions task</td>
<td>45</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Example of the Tower of Hanoi and Wisconsin Card Sorting tests</td>
<td>51</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Correlation of autistic symptoms in monozygotic and dizygotic twins</td>
<td>59</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Depiction of cytoarchitectonic regions of interest in autism across the entire human genome</td>
<td>61</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Diagram of chromosome 7 for a multiplex family</td>
<td>63</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Diagram of small interstitial deletion on chromosome 2 of a male with high-functioning autism</td>
<td>64</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Bar graph depicting reduced size of area dedanta across age in autistic subjects</td>
<td>83</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Best fit curve of meta-analysis of 15 studies depicting head circumference of autistic subjects across age</td>
<td>87</td>
</tr>
<tr>
<td>Figure 2.7</td>
<td>Depiction of differences in functional connectivity between autistic and typically developing subjects</td>
<td>90</td>
</tr>
<tr>
<td>Figure 2.8</td>
<td>Response of a single mirror neuron in area F5 of a Macaque Monkey</td>
<td>99</td>
</tr>
<tr>
<td>Figure 2.9</td>
<td>Recording of mirror neurons in area PF of Macaque Monkey which respond to specific intentions</td>
<td>103</td>
</tr>
<tr>
<td>Figure 2.10</td>
<td>Example of how intentions have been measured in humans</td>
<td>104</td>
</tr>
<tr>
<td>Figure 2.11</td>
<td>Mu wave suppression using EEG when autistic subjects observed a moving hand</td>
<td>107</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Screenshot of the four hand actions participants viewed</td>
<td>123</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Example of premotor activation in both groups</td>
<td>129</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Example of difference in premotor activation of both groups</td>
<td>132</td>
</tr>
<tr>
<td>Figure 3.4</td>
<td>Overall difference in between groups activation for both groups</td>
<td>132</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Overall functional connectivity for both groups when seeding premotor cortex</td>
<td>155</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Overall functional connectivity for both groups when seeding anterior cingulate cortex</td>
<td>157</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>Between groups differences in functional connectivity when seeding premotor cortex</td>
<td>159</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>Bar graph of Mean Fishers Z transformed scores for functional connectivity between premotor cortex and inferior parietal lobule, and premotor cortex and insula</td>
<td>159</td>
</tr>
<tr>
<td>Figure 5.1</td>
<td>Depiction of seed points for fibre reconstruction</td>
<td>177</td>
</tr>
<tr>
<td>Figure 5.2</td>
<td>Overall differences in fractional anisotropy and radial diffusivity between the groups</td>
<td>182</td>
</tr>
</tbody>
</table>
List of Tables

<p>| Table 1.1 | DSM IV Diagnostic Criteria for Autistic Disorder | 10 |
| Table 1.2 | DSM IV Diagnostic Criteria for Asperger Syndrome | 12 |
| Table 1.3 | Gillberg and Gillberg (1989) Diagnostic Criteria for Asperger Syndrome | 13 |
| Table 1.4 | DSM V Diagnostic Criteria for Autism Spectrum Disorders | 14 |
| Table 3.1 | Participant characteristics for both groups | 122 |
| Table 3.2 | Co-ordinates and activation of hypothesized regions for both groups | 128 |
| Table 3.3 | Co-ordinates and activation of other significant regions for both groups | 130 |
| Table 3.4 | Summary of co-ordinates which were significantly different between the groups | 131 |
| Table 4.1 | Summary of co-ordinates demonstrating significant functional connectivity with right premotor cortex for both groups | 154 |
| Table 4.2 | Summary of co-ordinates hypothesized to demonstrate significant functional connectivity with right premotor cortex | 155 |
| Table 4.3 | Summary of co-ordinates demonstrating significant functional connectivity with right anterior cingulated cortex for both groups | 156 |
| Table 4.4 | Summary of co-ordinates that demonstrated significantly different functional connectivity with the premotor cortex between the groups | 158 |
| Table 5.1 | Mean and standard deviations for fractional anisotropy and volume ratio for reconstructed coordinates | 180 |
| Table 5.2 | Summary of voxels demonstrating reduced fractional anisotropy, and increased radial diffusivity in participants with autism | 183 |</p>
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-D</td>
<td>Autistic Disorder</td>
</tr>
<tr>
<td>ACC</td>
<td>Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>AD</td>
<td>Axial Diffusivity</td>
</tr>
<tr>
<td>ADHD</td>
<td>Attention Deficit Hyperactivity Disorder</td>
</tr>
<tr>
<td>APA</td>
<td>American Psychiatric Association</td>
</tr>
<tr>
<td>AS</td>
<td>Aspergers Syndrome</td>
</tr>
<tr>
<td>ASD</td>
<td>Autism Spectrum Disorder</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
</tr>
<tr>
<td>CB</td>
<td>Cingulum Bundle</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>CNV</td>
<td>Copy Number Variation</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebro-spinal fluid</td>
</tr>
<tr>
<td>DF</td>
<td>DeFries-Fulker extreme analysis</td>
</tr>
<tr>
<td>DSM</td>
<td>Diagnostic and Statistical Manual for Mental Disorders</td>
</tr>
<tr>
<td>DTAP</td>
<td>Diptheria-Tetanus-acellular Petussis</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
</tr>
<tr>
<td>DZ</td>
<td>Dizygotic Twin</td>
</tr>
<tr>
<td>E-S theory</td>
<td>Empathizing-Systemizing theory</td>
</tr>
<tr>
<td>ED</td>
<td>Executive Dysfunction</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalograph</td>
</tr>
<tr>
<td>EMB</td>
<td>Extreme Male Brain theory</td>
</tr>
<tr>
<td>EPI</td>
<td>Echo-planar Imaging</td>
</tr>
<tr>
<td>FA</td>
<td>Fractional Anisotropy</td>
</tr>
<tr>
<td>FACT</td>
<td>Fiber Assignment by Continuous Tracking</td>
</tr>
<tr>
<td>FC</td>
<td>Functional Connectivity</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of View</td>
</tr>
<tr>
<td>FWE</td>
<td>Family Wise Error</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-Aminobutyric Acid</td>
</tr>
<tr>
<td>HFA</td>
<td>High Functioning Autism</td>
</tr>
<tr>
<td>HFA/AS</td>
<td>High functioning autism and Aspergers Syndrome Group</td>
</tr>
<tr>
<td>ICD</td>
<td>International Classification of Diseases</td>
</tr>
<tr>
<td>ID</td>
<td>Intellectual Disability</td>
</tr>
<tr>
<td>IFG</td>
<td>Inferior Frontal Gyrus</td>
</tr>
<tr>
<td>IMGSAC</td>
<td>International Molecular Genetic Study of Autism Consortium</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>IPL</td>
<td>Inferior Parietal Lobule</td>
</tr>
<tr>
<td>IQ</td>
<td>Intelligence Quotient</td>
</tr>
<tr>
<td>LCR</td>
<td>Low Copy Repeat regions</td>
</tr>
<tr>
<td>LT</td>
<td>Likelihood Threshold Modelling</td>
</tr>
<tr>
<td>MD</td>
<td>Mean Diffusivity</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor Evoked Potential</td>
</tr>
<tr>
<td>MMR</td>
<td>Measles, Mumps and Rubella vaccination</td>
</tr>
<tr>
<td>MN</td>
<td>Mirror Neuron</td>
</tr>
<tr>
<td>MZ</td>
<td>Monozygotic Twin</td>
</tr>
<tr>
<td>PDD</td>
<td>Pervasive Developmental Disorder</td>
</tr>
<tr>
<td>PDD-NOS</td>
<td>Pervasive Developmental Disorder not otherwise specified</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PLIC</td>
<td>Posterior Limb of the Internal Capsule</td>
</tr>
<tr>
<td>PMC</td>
<td>Premotor Cortex</td>
</tr>
<tr>
<td>RD</td>
<td>Radial Diffusivity</td>
</tr>
<tr>
<td>REST</td>
<td>RESTing State fMRI Data Analysis Toolkit</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>RRBS</td>
<td>Restricted and Repetitive Behaviour Symptoms</td>
</tr>
<tr>
<td>SAT</td>
<td>Social Attribution Task</td>
</tr>
<tr>
<td>SIA</td>
<td>Special Interest Area</td>
</tr>
<tr>
<td>SLF</td>
<td>Superior Longitudinal Fasciculus</td>
</tr>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphisms</td>
</tr>
<tr>
<td>STS</td>
<td>Superior Temporal Sulcus</td>
</tr>
<tr>
<td>TBSS</td>
<td>Tract Based Spatial Statistics</td>
</tr>
<tr>
<td>TD</td>
<td>Typically Developing</td>
</tr>
<tr>
<td>TMS</td>
<td>Transcranial Magnetic Stimulation</td>
</tr>
<tr>
<td>TOHT</td>
<td>Tower of Hanoi task</td>
</tr>
<tr>
<td>TOM</td>
<td>Theory of Mind</td>
</tr>
<tr>
<td>TS</td>
<td>Gilles de la Tourette’s Syndrome</td>
</tr>
<tr>
<td>VAERS</td>
<td>Vaccine Adverse Events Reporting System</td>
</tr>
<tr>
<td>VR</td>
<td>Volume Ratio</td>
</tr>
<tr>
<td>WCC</td>
<td>Weakened Central Coherence theory</td>
</tr>
<tr>
<td>WCST</td>
<td>Wisconsin Card Sorting test</td>
</tr>
<tr>
<td>WM</td>
<td>White Matter</td>
</tr>
</tbody>
</table>
Chapter 1: Autism
1.1 Introduction

For over 1000 years, people with severe social and communication difficulties have been observed in folklore, religious texts, and clinics. In 1943, Leo Kanner first described autism as a disorder with a basis in social and communication difficulties. Around the same time, Hans Asperger described a similar condition. Today, the DSM V conceptualizes autism as a developmental disorder that encompasses two areas of deficit: social and communicative abilities, and repetitive and restricted behaviours, interests and activities. Autism is considered to exist on a spectrum, reflecting the heterogeneous profile of symptoms and differences in severity between cases. Previously, the DSM IV recognized three subtypes of the disorder; autistic disorder (Kanner’s autism), high-functioning autism, and Asperger’s Syndrome. The basis for subtypes in this condition remains an ongoing question.

This chapter will provide a critical overview of the nature of autism spectrum disorders, with a review of their history, diagnosis, prevalence, comorbidities, symptoms, and cognitive profile. The key argument throughout this review is that diagnosis of autism spectrum disorders, and research into their key symptoms and cognitive basis has been impeded by the subjective and multi-dimensional nature of its core symptoms. This will be argued by highlighting the lack of clarity in diagnosing autism spectrum disorders, the subsequent heterogeneity of symptom expression, and limitations of cognitive theories to explain the autism profile. Thus, a key research priority is identification of biological antecedents that can help explain the major symptoms of autism.
1.2 Autism

1.2.1 History

Although autism was first conceptualized in the early 20th century, accounts of people who experienced marked social and communication difficulties date back over 1000 years. The explanation of these difficulties has been consistent with the prevailing views of the time. In the 10th century, folklore stories from Scandinavia, Germany and the United Kingdom tell of ‘changeling children’. According to this myth, young children were stolen by fairies, leaving behind an unresponsive, emotionless child known as a changeling. The depiction of a changeling as socially aloof, with difficulty coordinating motor actions has led some authors to speculate that this myth may have arisen from children who displayed autistic behaviours (Wing & Potter, 2002; Wolff, 2004). However, changeling children have not been exclusively linked to autism, with other authors attributing the changeling myth to developmental conditions such as mental retardation (Goodey & Stainton, 2001).

Writings by Saint Francis of Assisi dating back to the 11th century chronicle his followers, one of which was Brother Juniper. From these writings, Brother Juniper appears to demonstrate autistic characteristics including stubbornness, naiveté, and a lack of social intuition and common sense. However, this odd presentation was attributed to his ‘saintliness’ at the time. Brother Juniper followed the Franciscan precepts literally, to the point of taking off his clothes as a charity to beggars (Wing & Potter, 2002). Today, literal interpretation of metaphors, innuendo and figures of speech are considered key characteristics of Asperger’s Syndrome (AS) (Attwood, 2007), leading some
authors to speculate Brother Juniper may meet the criteria for this condition today (Wing, 1997).

In 1798, French Physician Jean Itard described in detail a case of *enfant sauvage* (a child raised in the wild by animals) known as Victor, ‘the wild boy of Aveyron’. In the five years Itard spent attempting to ‘humanize’ Victor; he described symptoms in the child that were similar to current conceptualizations of autism. Victor demonstrated restricted language and communicative abilities, an expressionless gaze, poor imitative abilities and a great sense of order, which bares some similarity to the presentation of autism (Wing & Potter, 2002). However, Victor’s presentation was likely influenced by a lack of socialization and parenting in critical developmental periods.

In the 19th century, two important psychiatrists published books that would later be highly influential on Kanner and Asperger. Firstly, in 1809 John Haslam published ‘Observations on Madness and Melancholy’, where he described a seven year old boy who was developmentally delayed in motor and communication abilities. In addition, he had a number of obsessive interests such as toy soldiers and church, and referred to himself in the third person. Haslam attributed this to possible brain abnormalities, an important insight at the time. Secondly, in 1879 Henry Maudsley published ‘The Pathology of the Mind’. This title includes a description of a 13 year old boy who may now meet the criteria for AS.

It is important to recognize that neither of these important historical figures made use of the term ‘autism’ (Wolff, 2004). It wasn’t until 1911, when Swiss psychiatrist Eugen Bleuler first used the term ‘Autism’, derived from the Greek word *auto*, (meaning self). Autism was first used to describe a symptom of
schizophrenia that involved a tendency to become preoccupied with one’s own thoughts, and neglect the external world (Fombonne, 2003). According to Bleuler, this led to withdrawal from socializing with other people, and an inability to form normal relationships. This could be distinguished from normal autism; the ability to conjure up fantasy’s, but separate them from reality.

In 1947, Gardner Murphy was among the first to extensively research autism, but his definition differed from Bleuler. Murphy considered autism to be the directing of cognitive processes toward satisfaction of needs. However, in 1951 Bleuler pointed out that autism is characterized more by problems in cognitive processing. In this early part of the 20th century, autism was not considered to be a disorder on its own.

In 1943, Austrian American Leo Kanner published ‘Autistic disturbances of affective contact’, a paper widely cited as the first to consider autism as a distinct disorder. Based upon 11 children (8 males, 3 females), this paper outlined a behaviour pattern he referred to as early infantile autism. In these children, Kanner observed abnormal speech, echolalia (repeating the vocalizations of others), interpretation of language literally, communication deficits and repetitive behaviours (Wolff, 2004). Kanner believed early infantile autism to be genetic in origin, a prediction that stands today. However the prominence of psychodynamic theory at the time meant that autism was typically attributed to parental attitudes such as the Refrigerator Mother theory (Rajendran & Mitchell, 2007), and personality (Wing & Potter, 2002). The children described in Kanner (1943) formed the basis for what is recognized as autism today.

In the same year, Hans Asperger submitted his doctoral thesis entitled ‘Autistic psychopathology in childhood’. However, it wasn’t published until 1944
Asperger described four male adolescents who were naïve and inappropriate in social interaction, displayed intense interest in specific areas, had good speech but poor body language and demonstrated poor motor coordination (Wing, 1997). Initially the work of Asperger went largely unnoticed in English speaking cultures. This was attributed to it only being published in German, and his work being less systematic than Kanner’s. Of interest, neither Asperger nor Kanner mentioned one another in their papers. This was despite the two being born in the same country, using the same term to describe the symptoms, and writing their papers at the same time (Lyons & Fitzgerald, 2007). It is unclear whether these two authors had any contact prior to publishing their papers.

Among Kanner’s most important contributions was his recognition that autism is distinct from schizophrenia (Volkmar, 2007). However, similarly to his failure to mention Asperger, Kanner made no reference to Bleuler’s earlier use of the term autism to describe a symptom of schizophrenia (Lyons & Fitzgerald, 2007). Although Kanner considered autism as being distinct from schizophrenia in his original paper, this was not empirically demonstrated until 1971. A London based psychiatrist by the name of Israel Kolvin (1971) conducted a series of clinical studies, and demonstrated autism was distinct from schizophrenia in terms of clinical features, course and family history.

Over the past 50 years, there have been a number of major developments in autism research. Kanner and Eisenberg (1956) published the first diagnostic criteria for early infantile autism. This criterion specified aloofness and indifference to others, repetitive routines, and an onset before 24 months of age (Wing & Potter, 2002). However, it was becoming clear that Kanner’s autism and
Asperger’s children had many symptoms that overlapped (Wing, 1997). Consequently, Wing and Gould (1979) changed the approach to research on autism by developing the spectrum of autistic disorders (ASD). The autism spectrum specified a triad of key symptoms; social deficits, communicative deficits and obsessive interests/routine behaviours. Each of these deficits could occur with varying severity and in different combinations (Wing & Potter, 2002). Thus, autism was viewed as a manifestation of any number of combinations of these three symptom clusters.

In 1980, the third version of the Diagnostic and Statistical Manual of Disorders (DSM III; American Psychiatric Association (APA), 1980) was released, and was the first version to recognize autism as a distinct disorder. Autism came under the general bracket of Pervasive Developmental Disorders (PDD); a term used to describe the developmental nature of the symptoms and pervasive deficits they caused. The DSM III subdivided PDD into five subtypes; however this was reduced to two in the revised third edition (DSM III-R, 1987). Autism was also recognized for the first time in the ninth version of the International Classification of Diseases (ICD-9, 1975). At this point, the children described by Asperger were not considered to be diagnostically separate from those described by Kanner.

In 1981, Lorna Wing revisited the work of Asperger, and was the first to describe AS as a separate disorder. This paper, along with Uta Frith’s translation of Asperger’s original thesis into English in 1991 were important in drawing attention to AS in the English speaking world (Wing, 2005). Wing (1981) observed that children with severe autistic symptoms could move along the autism spectrum as a result of early diagnosis and effective interventions.
The degree of handicap experienced as a function of autistic subtype is depicted in Figure 1.1. Wing’s (1981) and Frith’s (1991) papers provided the impetus for AS to be recognized as a disorder on the autism spectrum in the DSM IV (American Psychiatric Association (APA), 1994). The DSM V (2013), along with the ICD-10 provides the current conceptualization and understanding of ASD.

Figure 1.1: The degree of handicap experienced as a function of the subtype of autism on the spectrum. The ‘Triad’ refers to Wing and Gould’s (1979) patients who experienced deficits in the triad of symptoms. ‘Kanner’ refers to Leo Kanner’s ‘early infantile autism’ cases with severe to moderate retardation. ‘Asperger’ refers to Hans Asperger adolescent cases with autistic symptoms, and ‘DAMP’ (deficits in attention, motor control and perception) refers to Gillberg’s children (DAMP is not well accepted outside of Scandinavia; Gillberg & Gillberg, 1989).

1.2.2 Definition and Diagnosis

At present, autism is conceptualized as a genetic, neurodevelopmental condition that impact behaviour in a three domains; social development, communicative abilities and obsessive interests/routine behaviour (Baron-Cohen & Belmonte, 2005). The diagnosis of autism is in a transitional phase with the recently released DSM V. However, the recent changes have attracted some criticism (Wing, Gould & Gillberg, 2011). For this reason, this section will consider both the criteria stipulated by the DSM IV and V, and the challenges inherent with developing reliable diagnostic criteria for ASD.
According to the DSM IV-TR (APA, 2000), the two major ASD are Autistic Disorder (A-D) and AS. A subtype of A-D where intellectual functioning is not impaired is high-functioning autism (HFA). ASD are one of a number of disorders that belong to a group known as PDD. The core features of PDD are an early developmental onset of symptoms, and pervasive deficits in social and communication abilities (White, Keonig & Scahill, 2006). According to the DSM IV, PDD also include pervasive developmental disorder not otherwise specified (PDD-NOS), child disintegrative disorder and Rett’s Disorder.

A-D is diagnosed on the basis of abnormalities in the triad of behavioural domains specified by Wing and Gould (1979). Diagnosis of A-D requires two or more social impairment symptoms, and one or more communication deficits and restricted behaviour symptoms (Baron-Cohen, Wheelwright, Robinson & Woodbury-Smith, 2005). Overall, a case must demonstrate six or more of the 12 symptoms to be diagnosed with A-D (Tryon, Mayes, Rhodes & Waldo, 2006). In addition, a case must demonstrate a delay in the onset of language abilities, and not demonstrate abilities more characteristic of the other PDD. The full DSM IV criterion is provided in Table 1.1.

If a case has an IQ above 70 they are considered to have HFA, a form of A-D were intelligence does not demonstrate impairment. Research has demonstrated that the higher IQ of people with HFA leads to better social and adaptive skills (Koyama et al., 2007), and a more promising prognosis than those with A-D. Amongst those with autism, approximately 70% of cases are diagnosed with A-D, whilst the remaining 30% are considered high functioning (Ghaziuddin, Ghaziuddin & Greden, 2002). It must be recognized that although
HFA is a commonly used term for those on the autism spectrum who do not demonstrate intellectual impairment, it is not a recognized diagnosis.

Table 1.1
Diagnostic criteria for Autistic Disorder as specified by the DSM IV (1994)

A. A total of six (or more) items from (1), (2), and (3), with at least two from (1), and one each from (2) and (3)

(1) qualitative impairment in social interaction, as manifested by at least two of the following:

(a) marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction
(b) failure to develop peer relationships appropriate to developmental level
(c) a lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest)
(d) lack of social or emotional reciprocity

(2) qualitative impairments in communication as manifested by at least one of the following:

(a) delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)
(b) in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
(c) stereotyped and repetitive use of language or idiosyncratic language
(d) lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level

(3) restricted repetitive and stereotyped patterns of behavior, interests and activities, as manifested by at least two of the following:

(a) encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
(b) apparently inflexible adherence to specific, nonfunctional routines or rituals
(c) stereotyped and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole-body movements)
(d) persistent preoccupation with parts of objects

B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years: (1) social interaction, (2) language as used in social communication, or (3) symbolic or imaginative play

C. The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder.

Although the DSM IV provides a useful guide for practitioners to diagnose autism, it is not all encompassing. Within the communication deficits section, the DSM IV does not consider some of the subtle deficits in language
such as pragmatics, semantics, phonology and syntax. Moreover, the section on restricted, repetitive and stereotyped patterns of behaviour fails to consider some of the sensory abnormalities experienced by those with autism, such as hypo or hyper-sensitivity to stimuli.

Similarly to HFA, diagnosis of AS is made on the grounds of deficits in the triad of abilities, and an IQ above 70. Although there is debate as to whether these subtypes can be differentiated, there are three diagnostic criteria in the DSM IV that discriminate AS from HFA. Firstly, AS cases need to demonstrate fewer than six of the 12 symptoms, as opposed to six or greater in the case of HFA. Secondly, people with AS are diagnosed on the basis of a history of normal language development (Koyama et al., 2007), defined as expression of single words by age 2 and communicative phrases by age 3 (DSM IV; APA, 2000). Thirdly, for AS, qualitative impairments in communication are not required (Baron-Cohen et al., 2005). It is important to note that if a case demonstrates normal language acquisition, but more than six of the 12 symptoms, they still qualify for a diagnosis of AD. The DSM IV criteria for AS is presented in Table 1.2.

The criteria for diagnosing AS outlined by the DSM IV has been criticised as being too narrow (Baron-Cohen et al., 2005). More specific criteria identifies deficits that are present in A-D but absent in AS. For instance, Eisenmajer et al., (1998) notes that AS is generally not characterized by deficits in sustaining conversation, cognitive development, age appropriate self-help skills and adaptive behaviour, despite these being recognized in the DSM IV. Moreover, despite the DSM IV diagnosing AS on the basis of non-delayed language development, it fails to consider some of the speech and language
abnormalities present in AS (Attwood, 2007). These abnormalities include flat intonation and odd vocal pitch (Gillberg & Gillberg, 1989), and repetitive speech, idiosyncratic words, and pedantic monologues on topics of interest (Eisenmajer et al., 1996).

Table 1.2
Diagnostic criteria for Asperger Syndrome as specified by the DSM IV (1994)

- **A.** Qualitative impairment in social interaction that could be manifested by at least two of these criteria:
  1. Marked impairment in the use of multiple nonverbal behaviours such as facial expressions, body posture, and gestures in social interaction.
  2. Failure to develop appropriate peer relationships.
  3. Lack of spontaneous seeking of sharing enjoyment and interests of others.
  4. Lack of social and emotional reciprocity.

- **B.** Restricted repetitive and stereotyped patterns of behaviour, interests and activities as manifested by one of these criteria:
  1. Abnormal intensity or focus on one or more stereotyped and restricted patterns of interest.
  2. An apparent inflexible adherence to specific non-functional routines or rituals.
  3. Repetitive and stereotyped motor mannerisms.
  4. Persistent preoccupation with parts of objects.

- **C.** Impairment in social, occupational, or other important areas of functioning are clinically significant.

- **D.** No significant general delay in language.

- **E.** Cognitive development and age appropriate self-help skills and adaptive behaviour are not clinically significantly delayed.

- **F.** Does not meet criteria for another pervasive developmental disorder or schizophrenia.

Consequently, Gillberg and Gillberg (1989) outlined specific criterion that is more sensitive to diagnosis of AS (Table 1.3). This criteria specifies delayed spoken language as a possible symptom of AS, in addition to recognizing more subtle deficits such as odd speech prosody. Gillberg and Gillberg (1989) also consider a number of deficits that are more characteristic of AS than autism, such as motor clumsiness and gauche body language.
<table>
<thead>
<tr>
<th>Diagnostic criteria for Asperger Syndrome as specified by Gillberg and Gillberg (1989)</th>
</tr>
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<tbody>
<tr>
<td><strong>Severe impairment in reciprocal interactions (at least 2 of the following)</strong></td>
</tr>
<tr>
<td>inability to interact with peers</td>
</tr>
<tr>
<td>lack of desire to interact with peers</td>
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<tr>
<td>lack of appreciation of social cues</td>
</tr>
<tr>
<td>socially and emotionally inappropriate behaviour</td>
</tr>
<tr>
<td><strong>All absorbing narrow interest (at least one of the following)</strong></td>
</tr>
<tr>
<td>exclusion of other activities</td>
</tr>
<tr>
<td>repetitive adherence</td>
</tr>
<tr>
<td>more rote than meaning</td>
</tr>
<tr>
<td><strong>Impositions of routines and interests (at least one of the following)</strong></td>
</tr>
<tr>
<td>on self, in aspects of life</td>
</tr>
<tr>
<td>on others</td>
</tr>
<tr>
<td><strong>Speech and language problems (at least three of the following)</strong></td>
</tr>
<tr>
<td>delayed development</td>
</tr>
<tr>
<td>superficially, perfect expressive language</td>
</tr>
<tr>
<td>formal, pedantic language</td>
</tr>
<tr>
<td>odd prosody, peculiar voice characteristics</td>
</tr>
<tr>
<td>impairments of comprehension including misinterpretation of literal/implied meanings</td>
</tr>
<tr>
<td><strong>Nonverbal communication problems (at least one of the following)</strong></td>
</tr>
<tr>
<td>limited use of gestures</td>
</tr>
<tr>
<td>clumsy/gauche body language</td>
</tr>
<tr>
<td>limited facial expressions</td>
</tr>
<tr>
<td>inappropriate expression</td>
</tr>
<tr>
<td>peculiar, stiff gaze</td>
</tr>
<tr>
<td>Motor clumsiness</td>
</tr>
<tr>
<td>poor performance on a neuro-developmental examination</td>
</tr>
</tbody>
</table>

The DSM IV also outlines criteria to distinguish ASD from the remaining PDD. People with PDD-NOS have similar social impairments to ASD, but do not have severe enough communicative deficits or stereotyped behaviours to meet the diagnostic criteria for autism. Childhood disintegrative disorder involves similar social and motor deficits to ASD, but is distinguished by a late onset which follows a 2 to 4 year period of normal development. Rett’s Disorder involves social deficits and impaired motor skills, but can be distinguished from ASD by predominantly afflicting females, causing increasingly severe mental retardation, and an onset after a period of normal development.
Table 1.4. Diagnostic criteria for Autism Spectrum Disorders as specified by the DSM V (2013)

A. Persistent deficits in social communication and social interaction across contexts, not accounted for by general developmental delays, and manifest by all 3 of the following:

1. Deficits in social-emotional reciprocity; ranging from abnormal social approach and failure of normal back and forth conversation through reduced sharing of interests, emotions, and affect and response to total lack of initiation of social interaction.
2. Deficits in nonverbal communicative behaviors used for social interaction; ranging from poorly integrated-verbal and nonverbal communication, through abnormalities in eye contact and body-language, or deficits in understanding and use of nonverbal communication, to total lack of facial expression or gestures.
3. Deficits in developing and maintaining relationships, appropriate to developmental level (beyond those with caregivers); ranging from difficulties adjusting behavior to suit different social contexts through difficulties in sharing imaginative play and in making friends to an apparent absence of interest in people.

B. Restricted, repetitive patterns of behavior, interests, or activities as manifested by at least two of the following:

1. Stereotyped or repetitive speech, motor movements, or use of objects; (such as simple motor stereotypies, echolalia, repetitive use of objects, or idiosyncratic phrases).
2. Excessive adherence to routines, ritualized patterns of verbal or nonverbal behavior, or excessive resistance to change; (such as motoric rituals, insistence on same route or food, repetitive questioning or extreme distress at small changes).
3. Highly restricted, fixated interests that are abnormal in intensity or focus; (such as strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interests).
4. Hyper-or hypo-reactivity to sensory input or unusual interest in sensory aspects of environment; (such as apparent indifference to pain/heat/cold, adverse response to specific sounds or textures, excessive smelling or touching of objects, fascination with lights or spinning objects).

C. Symptoms must be present in early childhood (but may not become fully manifest until social demands exceed limited capacities)

D. Symptoms together limit and impair everyday functioning

In the year 2013, the DSM V was released, and made a number of significant changes to the diagnostic criteria of ASD. Firstly, social and communicative which have long been considered distinct symptom categories were collapsed into a single category (Table 1.4, part A). This has attracted controversy as some authors argue that social and communicative deficits are distinct genetically (Happè and Ronald, 2009). Second and more importantly, the DSM V has removed the sub categories (A-D and AS), in favour of one all encompassing category. In particular, this has attracted controversy as many individuals whom have been diagnosed with a particular subtype rely on their
diagnosis for particular medical or social services (Wing et al., 2011). Further, debate still remains whether A-D and AS can be differentiated diagnostically. Some of these issues are discussed in the next section.

1.2.3 Asperger’s syndrome and high functioning autism

As reflected in the DSM V, there is growing consensus that no diagnostically relevant differences exist between AS and HFA, as the two disorders share many of the same traits (Leekham, Libby, Wing, Gould & Gillberg, 2000; Wing & Potter, 2002). However, clinicians have pointed out that there are differences in the presentation of the two disorders (Manjiviona & Prior, 1999). This has prompted research to investigate whether different profiles of symptoms exist for HFA and AS. Among the main areas focused upon are differences in language development (Howlin, 2003), cognitive abilities (Ozonoff, South & Miller, 2000) and motor functioning (Manjiviona & Prior, 1999).

The role of language development in AS and HFA is unclear and heterogeneous. Some individuals diagnosed with HFA who present with delays in language have been identified to have a symptom profile more consistent with AS (Manjiviona & Prior, 1999). Eisenmajer et al. (1998) studied 106 cases of people with ASD, of which 62 had delayed language development. Of these 62 participants with delayed language, 27 of these 62 were diagnosed with AS. Moreover, language delay is not a universal feature of A-D and HFA (Macintosh & Dissanayake, 2004). Eisenmajer et al. (1996) reported that 26% of cases diagnosed with AD did not have significant language impairment. These studies
demonstrate that despite the diagnostic criteria, there are AS cases with language disturbance, and A-D cases without language disturbance.

A recent hypothesis has been that people with AS demonstrate overall stronger verbal and language abilities as opposed to delay, whilst people with AD demonstrate stronger perceptual reasoning skills (Macintosh & Dissanayake, 2004). Koyama et al. (2007) support this hypothesis, in a study reporting that AS participants scored higher on verbal abilities than HFA participants on the Wechsler Adult Intelligence Scale Revised. However females made up 9.3% of the HFA participants in this study, which was not representative of distribution of females with autism in the general population (25-33%; Baron-Cohen & Belmonte, 2005). Given the relative strength of females on verbal tasks, this under-representation may have skewed the results. Currently support for this theory is mixed, with some studies identifying stronger perceptual abilities in HFA compared to AS (Ehlers et al., 1999; Iwanaga, Kawasaki & Tsuchida, 2004; Ozonoff, et al., 2000), whilst other studies find the opposite pattern (Manjiviona & Prior, 1999).

Kanner considered motor delays as a characteristic of AS that separated this disorder from his early infantile autism children. However, like language development and cognitive abilities, the profile of motor skills in HFA and AS cases has yielded unclear results. Rinehart et al. (2006) compared people with AS and HFA to controls on a task involving motor preparation, and report a delayed response for those with HFA (724 ms to move stylus) compared to AS (516 ms). However, the opposite pattern has been reported (Szatmari et al., 1990). Moreover, in more controlled research that has age-matched participants, no differences in motor abilities between HFA and AS participants has been reported.
In sum, there is not yet reliable evidence or diagnostic indicators that distinguish AS from HFA. However, unclear diagnostic criterion has made reliable findings in this area difficult. For instance, many participants with AS also meet the criteria for HFA, or had a past diagnosis of HFA, which has changed as they matured (Wing et al., 2011). Although it has been argued that research adhering to strict diagnostic criteria and adequate power has found similar cognitive profiles between AS and HFA (Ghaziuddin et al., 1994), it remains possible that there are multiple autism spectra (Amaral, 2011), or that these two disorders are characterized by distinct neurodevelopmental patterns (Lotspeich et al., 2004). Thus, although there is not yet reliable data that separates AS from HFA, it remains possible these disorders are distinct.

1.2.4 Prevalence

Using DSM IV criteria, ASD were long considered to be rare, afflicting approximately 5 in every 10,000 people, or 0.05% (Gillberg, Cederlund, Lamberg & Zeijlon, 2006, 0.6; Baron-Cohen, 2002). In a review of 43 studies across 14 English-speaking countries looking at subtypes of ASD, Fombonne (2005) estimated that approximately 13 in 10,000 (0.13%) have A-D, 2.6 in 10,000 (0.03%) have AS, 21 in 10,000 (0.21%) have PDD-NOS, and 2 in 10,000 (0.002%) have childhood disintegrative disorder. The overall estimate for individuals afflicted with some form of PDD is 58.7 per 10,000 (Chakrabarti & Fombonne, 2005). The prevalence of ASD has steadily increased over time, with more recent estimates suggesting ASD has increased from 0.9% of the population
(Center for Disease Control, 2009), to 2.0% (Blumberg et al., 2013). The steady increase of diagnosed ASD over the past 30 years is depicted in Figure 1.2.

![Rise in prevalence of autism spectrum disorder's from 1977 to 1994 in Sweden. The lower portion of the bars represents males, whilst the upper portion of the bars represents females (Gillberg, Cederlund, Lamberg & Zeijlon, 2006).](image_url)

Figure 1.2: Rise in the prevalence of autism spectrum disorder’s from 1977 to 1994 in Sweden. The lower portion of the bars represents males, whilst the upper portion of the bars represents females (Gillberg, Cederlund, Lamberg & Zeijlon, 2006).

The rising prevalence of ASD has led some commentators to claim there is an ‘autism epidemic’ (Newschaffer et al. 2007). Although it is not clear how much of this variance can be attributed to a genuine rise in autistic symptoms in the population, it has mostly been attributed to a broadening of the diagnostic criteria over the past 30 years (Wing & Potter, 2002). For example, diagnosis of autism in the DSM III (1980) required six mandatory criteria. Although the currently used DSM IV (1994) also requires six criteria, they are spread over more categories (Gernsbacher, Dawson & Goldsmith, 2005). Moreover the DSM III-R only had two subtypes of PDD whilst the DSM IV extended this to five. This includes AS which was not included in the DSM III (Gillberg et al., 2006). It is likely that more specific diagnostic tools and increased awareness of autism has contributed to a rise in prevalence.

Moreover, epidemiological studies have been criticized on a number of grounds. Firstly, there is a lack of universal agreement on diagnostic criteria for
ASD (Szatmari et al., 1989). Over the past 15 years, the DSM IV criteria for diagnosing autism has been criticised as being too restrictive. For example, it dictates that a diagnosis of AD should take precedence over AS if a case meets the DSM IV criteria for AD, even if the patient’s clinical profile is more consistent with AS (Attwood, 2007). The previously mentioned Gillberg and Gillberg (1989) criterion better represents the characteristics of AS (as specified by Asperger), and estimates the prevalence of PDD to be approximately 36 to 48 per 10,000 people (Ehlers & Gillberg, 1993). This is higher than estimates from the DSM IV, which clearly shows that differences between diagnostic tools have an impact upon prevalence rates. It is likely that the alterations to diagnosis outlined by the DSM V will also impact upon prevalence rates.

Epidemiological studies have also had substantial methodological differences, which may have contributed to a rise in prevalence of ASD. One of the biggest confounding variables is variation in sample size. Honda, Shimizu, Misumi, Niimi and Ohashi (1996) reported that the prevalence of autism was found to be over 10 per 10,000 people more often in sample sizes fewer than 50,000. In Figure 1.3, prevalence rates in autism are plotted against sample size for 36 studies, taken from Fombonne (2005). This figure demonstrates a negative relationship, where the prevalence rate decreases as sample size increases. Moreover differences in how studies recruit cases leads to different prevalence estimates. For instance, routine developmental checks of people in prevalence studies produce the highest rates of ASD (Wing & Potter, 2002). Epidemiological studies will benefit from standardized methods by which to conduct this research.
Recognition that ASD can co-occur with other disorders has contributed to increased sensitivity in diagnosis. Prior to when autism became recognized as a disorder in the 1970s, people who demonstrated social difficulties were diagnosed with an intellectual disability (ID). Shah, Holmes and Wing (1982) conducted a study of an institution for people with mental retardation ($N=771$) born between 1880 and 1964. They found that 286 individuals had severe social impairment, of which only 29 had a previous diagnosis of autism. Given that the diagnostic criterion for autism has since broadened (DSM-IV, 1994), it is likely that some of the 257 socially impaired patients may now meet the criteria for an ASD (Wing & Potter, 2002). Overall, the increasing prevalence of ASD is likely to be the result of broadening diagnostic criteria, methodological differences between studies, and increasing awareness of autism.
An important observation first noted by Asperger (1944) is that autistic symptoms occur more frequently in males than females. Recent studies have confirmed Asperger’s observation. Gillberg et al. (2006) demonstrated a male to female ratio of 2.8 to 1 for AD, and 10.8 to 1 for AS (N=546). This is consistent with other research that estimates the ratio of males to females with AD at approximately 3 to 1. However estimates of male to female ratio for AS are mixed. Some sources report a male to female ratio of AS to be approximately 4 to 1 (Attwood, 2007; Ehlers & Gillberg, 1993), whilst others support the assertion that the male to female ratio for AS is approximately 10 to 1 (Baron-Cohen & Belmonte, 2005; Lawson, Baron-Cohen & Wheelwright, 2004). Recent evidence indicates that using DSM IV criteria yields a male to female ratio of 4 to 1, whilst using Gillberg’s more stringent criteria yields a ratio of 10 to 1 (Khouzam, El-Gabalawi, Pirwani & Priest, 2004). However, there has been doubt cast upon the suitability of current criteria to diagnose autism in females.

1.2.5 Comorbidity

There is growing consensus that ASD and other PDD are disorders without precise boundaries, with overlapping symptoms not accounted for by diagnostic criteria (Mattila et al., 2010). Among the most frequent co-occurring disorders with ASD are syndromes causing ID, attention deficit hyperactivity disorder (ADHD), mood disorders such as depression and Tourette’s syndrome. However, research examining comorbidity between ASD and these disorders has been addressed infrequently (Gillberg & Billstedt., 2000).

Overall, ID is the most common comorbid diagnosis with ASD. Approximately 70% of people diagnosed with autism have an ID (Ghazziuddin,
et al., 2002). However, the prevalence of ID among those with autism is considered too high for a number of reasons. Firstly, Koegel et al. (1989) argue that many autistic children perform poorly on intelligence tests because they are not motivated to perform their best on these tests. Secondly, many children with autism can acquire intellectual skills, but fail to apply these in day-to-day life (Freeman & Van Dyke, 2006). Chakrabarti and Fombonne (2005) studied participants with PDD ($N=64$) and found the prevalence of ID was 29.8%, which would appear to support these claims of an exaggerated ID estimate. However, these authors fail to note that when this sample was sub-divided into participants with AD, the prevalence of ID among this sub-group was 66.7%.

There are two major causes of mental retardation that have been found to co-occur with autism. Firstly, Down syndrome is a chromosomal disorder caused by a third pairing of chromosome 21. The occurrence of Down syndrome in people with AD is estimated to be approximately 16%. However, when the effect of mental retardation by other causes is controlled for, the co-occurrence of autism and Down syndrome is not higher than expected by chance (Zafeiriou, Ververi & Vargiami, 2007). Secondly, Fragile X Syndrome is a genetic disorder caused by too many trinucleotide repeat expansions at Xq27.3, which leads to intellectual disability and social difficulties (Lenti, Peruzzi & Bianchini, 1995). Comorbidity rates between autism and Fragile X Syndrome range between 25-47% (Hooper et al., 2008). For instance, Bailey et al. (1998A) studied 57 participants with Fragile X Syndrome, and found that 14 (25.6%) met criteria for autism using the childhood autism rating scale. This scale isn’t equivalent to DSM IV criteria for AD however.
ADHD is characterized by inattention, hyperactivity and impulsivity (Corbett & Constantine, 2006), and is the most common comorbid diagnosis in children with autism (Gillberg & Billstedt, 2000). According to the DSM IV, autism and ADHD should not be diagnosed conjointly in an individual patient. Nevertheless, ADHD has been reported to be as high as 50% in ASD (Matson & Nebel-Schwalm, 2007). For example, Ghazziuddin, Weidmer-Mikhail and Ghazziuddin (1998) found that out of 20 participants aged between six and 12 diagnosed with autism, 10 also met the diagnostic criteria for ADHD. Despite the small sample size, this finding has since been replicated (Goldstein & Schwebach, 2004).

Among adults, depression is the most common comorbid disorder with autism, occurring in about 30 to 50% of adults. Ghazziuddin et al. (1998) found that in a sample of 15 autistic participants over 13 years of age, eight participants had a diagnosis of depression. However, with only 15 participants it is difficult to generalize these results. Wing (1981) described 34 adults with AS, and determined that 10 (29.4%) of these participants had a previous psychiatric diagnosis of depression. A number of other clinical studies report diagnosis of depression in those with autism (Clark, Little-Johns, Corbett & Joseph, 1989; Realmoto & August, 1991). Mood disorders are reported to be more common in AS than AD, occurring in about 2% of children with AD, and 30% of children with AS (Matson & Nebel-Schwalm, 2007).

Gilles de la Tourette’s Syndrome (TS) is a neuro-developmental disorder characterized by the presence of vocal and motor tics. TS and autism have a number of common symptoms including echolalia, obsessive compulsive behaviours and abnormal motor behaviours. Early research estimated TS to co-
occur with autism in approximately 30 to 50% of cases (Kerbeshian & Burd, 1986). However more recent research using specific diagnostic criteria has found this to be much lower. Baron-Cohen, Mortimore, Moriarty, Izaguirre and Robertson (1999) investigated 61 children with autism, and found four of these children (8.1%) also met DSM III-R criteria for TS as determined by a neuropsychiatrist, psychologist and specialist in TS diagnosis. Although more research is necessary, this prevalence estimated has been supported (Canitano & Vivanti, 2007).

1.3 Triad of Autistic Symptoms

There is considerable variability in symptom severity in ASD. Social deficits can range from complete disinterest in socializing to mild difficulties interpreting social cues, communication deficits can range from absence of language to minor pragmatic deficits in speech, and obsessive interests can range from occasional repetition of motor behaviours, to elaborate, compulsive rituals (Penn, 2006). Furthermore, the symptoms of autism are not as simple as the frequency or absence of a particular behaviour (Mundy & Crowson, 1997). For some individuals with autism, symptoms may only be expressed in specific social contexts, or expressed in contextually inappropriate ways (Wilkinson, 1998). Mindful of these complexities, this section will review the range of social, communication and restricted behaviour symptoms that characterize ASD.
1.3.1 Social Deficits

Problems with social functioning are the core deficit of autism, and a source of impairment regardless of cognitive or language skills (White, et al., 2007). If interventions are not made early in a child’s development, social deficits can be a key determinant of comorbidities such as depression (Bellini & Hopf, 2007). It is important to recognize there is currently no standardized test to determine overall social functioning (Attwood, 2007). Among the most pronounced social difficulties in ASD are problems with joint attention, eye contact, impulsivity and hyperactivity, imitation and empathy, social anxiety and problems developing relationships.

Difficulty sustaining or initiating joint attention is one of the most common deficits in autism (Delincolas & Young, 2007). Joint attention is a general skill that involves coordinating the attention of another person and environmental stimuli in a social exchange (Whalen & Schreibman, 2003). Joint attention can be a response (such as following the point of a parent’s finger), or an initiation (directing a friend by pointing). It requires an ability to interpret social cues, and is critical to interact with others (Constantino & Todd, 2000). Joint attention is a deficit relatively unique to ASD, as children with developmental and language delay have been found to respond appropriately to joint attention tasks more often than those with autism (Loveland and Landry, 2005).

Evidence of joint attention deficits in autism are well established (Sigman & Ungerer, 1984; Toth, Munson, Meltzoff & Dawson, 2006). Most research has focused on parental reports of their children from 12-18 months of age (Charman, 2003), or retrospective home-video analysis (Werner, Dawson, Osterling, &
Dinno, 2000). A study by Presmanes, Walden, Stone and Yoder (2007) was able to directly examine joint attention deficits without relying on retrospective evidence. The researchers examined 12-23 month old children with ASD and compared them to TD boys \( (N=35) \) by having research assistants code for these skills, and report the boys with ASD used significantly less of these skills.

A deficit in autism associated with joint attention is inappropriate eye contact during a social exchange (Bruinsma, Koegel & Koegel, 2004; Osterling, Dawson & Munson, 2002). Eye contact when engaged in conversation allows people to read others expression and respond appropriately (Attwood, 2007). Similarly to joint attention, impaired eye contact in a social exchange is relatively unique to ASD, with deficits being more pronounced than TD participants and those with mental retardation (Mundy, Sigman, Ungerer & Sherman, 1986).

More recent research has focused on exactly where those with autism focus their eyes during conversation. Klin, Jones, Schultz, Volkmar and Cohen (2002) used eye-tracking technology to compare TD and ASD participants \( (N=15 \) in each group) visual focus whilst watching a video of a conversation between two people. The results indicate that those with autism spent more time fixated upon objects unrelated to the conversation \( (M=9.6 \text{ seconds}) \) by comparison to controls \( (M=3.7s) \). The results of two participants are depicted in Figure 1.4. This finding explained a small amount of variance in reduced social adaptation \( (R^2=-0.25) \), and increased social disability \( (R^2=0.41) \). However, the group differences may be under-estimated, as 15.6% of visual frames had to be omitted in the autism group due to their visual focus being off the screen. Abnormal eye gaze is a well-established finding among participants with an ASD (Baron-Cohen, Wheelwright & Joliffe, 1997; Vlamings, Stauder, van Son & Mottron, 2005).
Those with autism can be impulsive and hyperactive. Hyperactivity is particularly prevalent in social situations, where they respond to people without considering the context or consequences. An individual with autism may act out with aggression if someone begins to annoy them, where such a response would ordinarily be inhibited. Impulsive behaviour is more likely among those with autism when under stress (Attwood, 2007). Although most findings on hyperactivity come from clinical case reports, research has demonstrated hyperactivity and impulsivity to be more common in autism. In a study of both TD and ASD participants with 31 participants in each group, Bradley and Isaacs (2006) report that 48% of the ASD group met the criteria for impulsivity and 35% hyperactivity, compared to 19% and 9% of participants in the TD group. It has recently been argued that impulsivity and hyperactivity in ASD likely represents co-morbidity with ADHD (Gargaro, Rinehart, Bradshaw, Tonge & Sheppard, 2011).
Deficits in imitation have been observed in autism. Imitation is an ability present in newborns that develops rapidly (Smith, Mirenda and Zaidman-Zait, 2007), and is a form of learning that allows a child to bypass trial and error (Iacoboni, 2007). In a meta-analysis of 18 studies of imitative abilities in autism, 14 identified deficits, which were mostly attributable to younger participants (Williams, Whiten & Singh, 2004). Several studies report that imitation abilities amongst participants with ASD tend to be better in structured settings, by comparison to naturalistic settings (for a review, see Vanvuchelen, Roeyers & Weerdt, 2011). For instance, a clinical record reported by Attwood (2007) describes a 21-month-old autistic case that could not follow a simple pat-a-cake game with his mother, where she would have to physically move his hands. Imitation deficits are more disturbed in ASD than developmentally delayed individuals (Rogers, Bennetto, McEvoy & Pennington, 1996), making it a deficit relatively unique to autism.

Disturbances in empathy can also be observed in participants with an ASD. Empathy is a construct that allows one to identify other people’s beliefs, desires and intentions, and respond to these mental states with an appropriate emotion (Sucksmith, Alison, Baron-Cohen, Chakrabarti & Hoekstra, 2013). In TD cohorts, this ability is remarkably intuitive (Baron-Cohen & Wheelwright, 2004). Impairments in empathy can lead to difficulties interpreting other people’s emotions, a tendency to make literal interpretations of expressions (such as ‘let’s hop on the scales’), difficulty following social rules (such as withholding comment on an overweight man’s size) and a failure to recognize that other people can help in problem solving (Attwood, 2007).
Empathy deficits in autism have been reported with a variety of methods. Baron-Cohen and Wheelwright (2004) compared TD and HFA participants ($N=90$ each) on the empathy quotient (a self-report inventory), and found TD participants scored higher ($M=42.1$) compared to HFA participants ($M=20.4$) out of a total score of 80. This finding has since been replicated in both male and female participants with an ASD (Sucksmith et al., 2013).

A more direct measure of empathy involves recognizing the emotional expression of others’ faces. Humphreys, Minshew, Leonard and Behrmann (2007) investigated individuals with HFA ($N=21$), and found 20% were impaired in recognizing faces of anger, 20% disgust and 5% happiness. This study did not measure visual attention, meaning it is possible these findings are also attributable to inappropriate eye contact. Although empathy deficits are common in ASD (Hobson, Ouston & Lee, 1988), some studies have reported no difficulties (Baron-Cohen, Jolliffe, Mortimore & Robertson, 1997; Schulte-Rüther et al., 2011).

One consequence of the impairments outlined above is that people with autism do not develop typical peer relationships and friendships. Friendship serves the function of affection, trust, intimacy and companionship (Bauminger, Shulman & Agam, 2004), and are critical to effective social development. Howlin, Mawhood and Rutter (2000) studied 19 individuals with an ASD and reported that 16% had one or more friends, 32% had an acquaintance with which they shared an interest, and 47% reported no friends. Although the ability of people with autism to relate to others improves during adolescence (Orsmond, Krauss & Seltzer, 2004), they still demonstrate difficulties developing relationships with people in adulthood.
People with autism also participate in less social events. Ormond et al. (2004) surveyed 407 individuals with autism, and found that the most common recreational activities of autistic people were independent tasks such as exercising (74.5%) or personal hobbies (41.3%). Only 20.9% socialized informally with friends, with low frequency of friends being significantly correlated to low participation in social activities ($R=.40$). A common misconception is that people with autism lack an interest in social interaction. It is important to recognize that many children with autism desire friendships and social activities, as they report feelings of loneliness, poor understanding of their loneliness and low quality of companionship (Bauminger & Kasari, 2000).

People with autism experience higher social anxiety and depression than the general population. Green, Gilchrist, Burton and Cox (2000) studied 20 males with AS, and found that 35% met the ICD-10 criteria for generalized anxiety disorder. Further, Bellini (2006) looked specifically at social anxiety in adolescents with an ASD, and reported that 49% of the autistic sample demonstrated clinically significant social anxiety. However, a problem with this study is participants were recruited through an invitation that specified the research was looking at social anxiety, which likely led to a biased sample. It is unclear whether social anxiety in autism is a direct consequence of the aforementioned social deficits, or an independent symptom in its own right.

1.3.2 Communication deficits

An important part of social behaviour is the ability to communicate effectively both verbally and non-verbally. Language is considered to be fundamental to expression of culture, intellect and abstract thinking (Wilkinson,
1998). For those with an ASD, the range of language skills is diverse. Severe cases can demonstrate almost complete absence of language or communicative abilities, whilst moderate cases can demonstrate well developed spoken and written syntax. The following section will address evidence for language deficits in four domains: pragmatics, semantics, phonology and syntax, whilst recognizing the role social deficits have in expression of language impairment.

Language pragmatics refers to context-appropriate use of language, where deficits impact the ability to apply language appropriately to social situations (Philofsky, Fidler & Hepburn, 2007). Pragmatic language abilities can overlap with the specific social skills required for joint attention, such as eye-contact and gaze. However, pragmatic deficits that do not overlap with these social deficits such as difficulty understanding body language, strange emotional interpretations and problems with humour are well documented in the literature (Dewey & Everard, 1974; Eisenmajer et al., 1998; Happé & Frith, 1996).

Some pragmatic language deficits observed in autism are not found in language disorders. Using audiotaped conversations, Eales (1993) found ASD participants (N=15) demonstrated significantly more inappropriate disruptions during conversation, more failures to respond during conversation and less conversation initiations than individuals with receptive language disorder (N=17). Loukusa et al. (2007) suggest pragmatic deficits stem from a failure to use contextual information, which emphasizes the subtle, social basis of language deficits in ASD. It is important to note that given the complexity of pragmatic communication skills, research to date has relied on non-standardized, observational methods (Philofsky et al. 2007).
Phonology refers to the rules governing the production of speech sounds (Wilkinson, 1998). In social contexts, phonology is intimately linked to the pragmatic rules that give words their contextual meaning. The most pronounced phonological difficulty among those with autism is **prosody**, which refers to the stress, intonation and rhythmic variables of speech. Those with an ASD can possess atypical speech prosody, in addition to difficulties interpreting the emotional valence of others' prosody (Hubbard & Trauner, 2007). Prosody has functions in grammar (using rising pitch to emphasize a question), pragmatics (emphasizing certain words to draw attention to important information) and affect (varying tone to indicate a certain mood) (Attwood, 2007).

Early phonological research reports autism being characterized by a ‘singsong’ vocal quality with a larger than normal range of pitch (Fay & Schuler, 1980). Supporting this view, Hubbard and Trauner (2006) utilized pitch measurement software, and found participants with AD had a larger pitch range than TD participants. It is important to note several clinicians describe ASD as being characterized by stiff, monotonous speech – which may suggest a general deficit in how to appropriately apply and interpret pitch (Wilkinson, 1998). In favour of this view, general abnormalities in the **stress** of words are reported as being the most common phonological deficit in ASD (Paul, Augustyn, Klin and Volkmar, 2005; Wilkinson, 1998). Thus, autism seems to be characterized by a general deficit in emotionally appropriate inflection and interpretation of speech.

Semantics refers to the rules that govern the meaning and concepts of words (Wilkinson 1998). Three semantic abnormalities commonly associated with autism are idiosyncratic language, neologisms and literal interpretations. Idiosyncratic language is when a child associates a word or phrase to a seemingly
unrelated object or event. Frith (1989) described a young autistic boy whose mother recited ‘Peter Peter pumpkin eater’ to him. While doing this, she once dropped a saucepan. As a result the young boy began calling anything resembling a saucepan a ‘Peter Eater’.

Neologisms refer to odd vocalizations that do not correspond to a particular word or object, but can nevertheless be ‘decoded’ by a parent as a request or indicative of a mood (Frith & Happé, 1994). Attwood (2007) reported a case of AS that referred to ice cubes as ‘water bones’. A limited body of research has identified neologisms and idiosyncrasies as being more prevalent in autism (Volden & Lord, 1991). However, misuse of language is not exclusive to autism. It is common for TD children to express neologisms and idiosyncratic language. What makes these characteristics atypical in ASD is their frequency, persistence and inappropriate use (Wilkinson, 1998).

Those with an ASD tend to interpret people literally. Attwood (2007) describes a case of a young girl whom he asked whether she could count to ten. She simply stated ‘yes’ and continued to play. Her semantic difficulty lies in the failure to recognize the question as also being a request to perform. Literal interpretation extends to metaphors, idioms and irony (Wang, Lee, Sigman & Dapretto, 2006). For example, expressions such as ‘I caught his eye’ can be interpreted literally. It is possible this deficit is linked to empathetic abnormalities, as they fail to understand what other people are thinking, and by extension the intent of their requests.

People with autism have difficulty with syntax. Although not consistently identified, abnormal use of pronouns is a symptom almost exclusively reported in autistic participants (Wilkinson, 1998). Lee, Hobson and Chiat (1994) compared
adolescents with A-D to TD participants on the use of pronoun comprehension. In this study the experimenter viewed a picture that the subject could not see, and then asked the autistic subject who was viewing the picture. Three of nine autistic participants made pronoun reversal errors (i.e. ‘I can’ instead of ‘you can’), whilst none of six TD participants made pronoun reversal errors.

Although the majority of autistic participants did not make this error, the teacher’s reports prior to this study indicated that the autism participants tended to have sporadic difficulties with pronoun production from day to day. For instance, the authors noted that one of their participants stated ‘thanks for seeing you’ (referring to himself) upon finishing testing. These post-research notes emphasize that syntax errors in autism are likely linked to a general difficulty understanding social pragmatics, particularly when under stress, or when information is ambiguous (Wilkinson, 1998).

Another syntactic deficit in ASD is echolalia, which refers to the repetition of a word or sentence spoken by another person, usually without communicative intent. Echolalia was identified by Kanner (1943) as being among the most common abnormalities of speech in those with an ASD. Like many symptoms of autism, echolalia is also common among TD children, with an age of onset of approximately 10 months old. What distinguish echolalic utterances in those with autism are there frequency, late onset, prolonged use and lack of communicative intent (Prizant & Rydell, 1984). It is possible echolalia functions as a stage between comprehension and production of language (Howlin, 1982), whereby those with autism fail to progress developmentally.
1.3.3 Restricted and repetitive behaviours

By comparison to the core social and communication deficits of autism, less research has been conducted on the Restricted and Repetitive Behaviour Symptoms (RRBS) (Baron-Cohen, 1989; Militerni, Bravaccio, Falco, Fico & Palermo, 2002). One reason is that the RRBS do not form a homogenous group as tidily as the social and communication deficits. RRBS not only impact behaviours, but also interests and activities (Szatmari et al., 2006). For the purpose of this section, RRBS will be used as a generic term to describe all forms of restricted behaviours, interests and activities. In ASD, there are five major areas of deficit; restricted interests, a preoccupation with the parts of objects rather than their whole function, inflexible adherence to routines and rituals, abnormal motor actions and abnormal response to sensory information.

Special Interest Areas (SIA) are common among participants with an ASD. SIA can be discriminated from a normal hobby by the abnormal intensity and focus in the interest, often on topics that may seem mundane, sterile or unusual to others (Frith, 1991). The prevalence of SIA in individuals with an ASD has been estimated to be as low as 31% (Kerbeshian, Burd & Fisher, 1990; Turner, 1999), and as high as 90% (Szatmari et al., 1989). Measures such as the Yale Special Interests Interview provide evidence those with an ASD have stronger SIA than TD individuals (South, Ozonoff & McMahon, 2007). The most common SIA among those with autism are animals and nature (such as dinosaurs), science (such as astronomy and the planets), public transport systems (such as memorizing train timetables) and art (such as drawing) (Attwood, 2007).

Although the precise reasons why SIA develop are complex, Winter-Messiers (2007) suggests social factors are partly responsible. For an individual
with an ASD, SIA are a source of reliability by comparison to the ‘confusing’ social world. Although SIA can aid in peer acceptance, they can also lead to teasing when compared to individuals who may have an abnormal passion for something more socially accepted such as football. Moreover, some individuals with an ASD demonstrate increased intelligibility and communicative skills when addressing an SIA. Clinical observations described those with autism as ‘little professors’ when addressing their SIA (Gillberg, 1991). This may lead to extraordinary performance in domains such as music (Asperger, 1944).

Those with an ASD can also exhibit inflexible adherence to rituals and routines. Wing and Gould (1979) found that elaborate routines and rituals are among the clearest discriminator between autistic and non-autistic individuals (94% of cases compared to 2% of TD cases). Firstly, a ritual is a behaviour performed repetitively in the same set manner. For example, a child with an ASD may spend hours lining up pieces of Lego. By comparison to TD children, autistic rituals are highly elaborate, and extend to activities that impact upon other people (Attwood, 2007).

Secondly, Routines are defined as customary forms of procedure. Turner (1999) describes an individual with an ASD who strictly adhered to a bus schedule, where any change to this schedule caused aggravation and stress. Furthermore, Happé and Frith (2006) describe a case that routinely stored his book collection in order, but was caused enormous discomfort when any book was misaligned. The large amount of distress toward a change in routine is rarely observed in TD children. Several studies using measures such as the Repetitive Behaviour Interview have demonstrated participants with an ASD take part in
more rigid routines than TD individuals (Bartak & Rutter, 1976; Lord & Pickles, 1996; South et al, 2007).

Routines in participants with ASD have also been distinguished from other disorders characterized by routine behaviour. Greaves, Prince and Evans (2006) compared ASD to Prader-Willi Syndrome; a genetic disorder characterized by ritualized behaviour, and reported that routines in ASD were motivated by a desire for order, rather than hoarding, which is typical of Prader-Willi Syndrome.

A characteristic of cognitive processing in ASD is a preoccupation with the individual components of an object, whilst de-emphasizing its entire function. This is generally considered a deficit of focus rather than attention, as persons with ASD becomes fixated upon small details of a task (Baron-Cohen, 2002). For instance, an autistic individual may listen to a piece of music, but be preoccupied with the pitch of each individual note rather than the flow of the song. This focus upon detail can be an important contributor to their routines and rituals, and can lead to a fragmented understanding of objects and their function. Bölte, Holtmann, Poustka, Scheurich and Schmidt (2007) found that HFA participants used Gestalt principles such as similarity ($\eta^2=0.66$), proximity ($\eta^2=0.11$) and closure ($\eta^2=0.12$) significantly less than TD participants (see Figure 1.5). Interestingly, the HFA group performed marginally better on block design ($\eta^2=0.03$) which is a task requiring attention to detail.
Some individuals with an ASD can still demonstrate a global understanding of phenomena, despite their focus on smaller detail. Mottron, Burack, Stauder and Robaey (1999) describe a savant draughtsman with autism who would begin drawing the local details first, with no particular sequence or plan. His art still demonstrated perfect global proportions, despite most conventional artists starting with the global details, and then proceeding to draw in the details sequentially. A focus on the smaller details (whether it be drawing or scientific theory) can help in developing expertise in a SIA (Attwood, 2007).

Although early reports of ASD describe their movement as being graceful and well-coordinated, individuals with an ASD can demonstrate motor anomalies. The prevalence of motor abnormalities is estimated at being between 50-80% (Gillberg, 1989; Manjiviona & Prior, 1995; Miyahara et al., 1997), and can be distinguished between abnormal movements and repetitive, intrusive tics.
Atypical movement amongst individuals with ASD is characterized by clumsy, poorly planned (motor apraxia) or poorly coordinated motor behaviour. A broad range of research has identified abnormal movement in ASD by comparison to TD participants (Green et al., 2002; Jansiewicz et al., 2006; Provost, Lopez & Heimerl, 2007; Rinehart et al., 2006), and covers a wide range of anomalies including unusual gait, difficulty synchronizing leg movement, postural stability problems, difficulty maintaining balance when placing one foot in front of the other, and problems with fine motor skills such as opening ones hands to catch a ball (Ozonoff et al., 2008). Another movement difficulty observed in ASD is hypotonia, which is reduced resistance during passive movement of the limbs (Ming, Brimacombe & Wagner, 2007).

The second type of motor abnormality observed in ASD is repetitive and intrusive tics and twitches. These can be elaborate motor repetitions such as hand flapping, twirling or hopping, or simple tics such as eye blinking, tongue protrusion and nose twitching (Attwood, 2007). Between 20 and 60% of those with an ASD develop tics (Hippler & Klicpera, 2004). Tics generally occur unconsciously, meaning they are difficult to inhibit (Ming et al., 2007). Most research on motor tics in ASD has come from individual case reports. Ringman and Jankovic (2000) reported autistic case ‘J.M’, whom had a tic of bilateral nasal contraction, as well as frequent touching of the opposite hand. As previously discussed, these motor tics can be so severe that the autistic case also meets the diagnostic criteria for TS.

Abnormal response to sensory stimuli is a key symptom of ASD. Despite sensory abnormalities often having a greater impact on day to day functioning than social and communication deficits, they are not considered a symptom that
diagnostically differentiates autism from other developmental disorders (Ozonoff & Rogers, 2005). An abnormal sensory response can be hyper or hypo sensitivity, and affects both proximal (olfactory, gustatory, pain, temperature tactile and vestibular) and distal (visual and auditory) senses. The nature of the abnormalities ranges from sensory distortions (such as ‘tuning out’ from sensory information) and difficulty identifying the source of sensory information (Bogdashina, 2003).

Some evidence for sensory anomalies has been reported by individuals with ASD themselves. Attwood (2007) describes two peculiar cases of autism that possessed sensory anomalies. Case 1 refers to a hypersensitive autistic woman who by smell alone was able to warn diners at a restaurant that their seafood was off and would make them ill, whilst case 2 describes a hyposensitive man that wore a t-shirt all night in near freezing temperatures whilst camping. Over 90% of those with an ASD have some form of sensory abnormality (Dunn & Bennett, 2002; Leekham, Nieto, Libby, Wing & Gould, 2007), with a range of approximately 42-88% of cases also constituting a sensory processing disorder (Tomchek & Dunn, 2007).

Further research has examined the breakdown of which sensory modality is most commonly afflicted in ASD. In a study of 33 children with some form of ASD, Leekham et al. (2007) reported that auditory (47%), tactile (47%) and olfactory/gustatory (47%) were the most common sense organ afflicted. Vision (35%) and pain (6%) were also prevalent in a minority of cases. This research did not specify the nature (such as hyper or hyposensitivity) of the abnormal sense responses. What is clear is that despite sensory abnormalities being among the most common deficits in autism, their expression is highly heterogeneous.
1.3.4 Relationship between the triad of symptoms

Since Wing and Gould introduced the triad of symptoms in 1979, a sensible explanation for their co-occurrence has proven difficult. Recent genetic research has made inroads regarding how related these three symptom clusters are. Two studies by Ronald, Happè & Plomin (2005) and Ronald et al. (2006) assessed 3,419 TD twin pairs aged between 7 and 9 on autistic symptoms. The first of these studies found that 59% of children who showed social impairments demonstrated only social impairments. Approximately 10% of all children demonstrated only social impairments, only communication impairments, or only RRBS. The second Ronald et al. (2006) study also investigated the relationship between the three domains, and found only modest relationships between social and communication deficits ($R=0.34$), social deficits and RRBS ($R=0.23$) and communication deficits and RRBS ($R=38$).

Happè et al. (2006) have made a preliminary finding that these three symptom domains can, and often do occur in isolation, where only a small amount of variance in the triad of symptoms overlap. However, it is important to note that their data relied upon parental reports of these domains in their children, which may be unreliable. Moreover, the triad of symptoms that characterize ASD may not be reflected accurately in a TD sample. Nevertheless it is important for the ASD literature to clarify the relationship between the symptom categories as it will have important consequences for understanding the basis of the disorder.
1.3.5 Conclusion

In summary, although the symptoms of autism are recognizable, their expression is heterogeneous. At its core, autism is characterized by social deficits in joint attention that can lead to general socially based difficulties. However, the specific social deficits in eye contact, empathy and impulsive behaviour can be expressed very differently. Communication deficits and RRBS are prevalent in almost all cases of ASD, but the nature of these difficulties can be subtle, contextual and vary greatly in severity. Although RRBS have received less attention in the literature, they can be a significant source of distress. The heterogeneity in the autism phenotype presents a unique challenge in explaining these symptoms.

1.4 Cognitive Theories of Autism

Early theories seeking to explain the basis of autism focused upon attachment to parents. For example, ‘the refrigerator mother’ theory suggested that an emotionless parenting style leads to the development of autism (Rajendran & Mitchell, 2007). Although these theories have since been discredited, present-day theories focus upon differences in cognitive processes between those with autism and TD individuals. Not surprisingly, given the heterogeneous profile of the disorder, there is no single cognitive theory that can adequately explain autism (Ronald, Happè & Plomin, 2005). Nevertheless, three broad theories argue that individuals with autism differ in three key areas of information processing; theory of mind, systemizing of information, and executive functioning.
1.4.1 Theory of mind hypothesis

Broadly, Theory Of Mind (TOM) refers to a cognitive ability developed in early childhood that allows people to attribute subjective mental states to oneself, and to others. This includes goals, desires, emotions and opinions (Colle, Baron-Cohen & Hill, 2007). In turn, this allows people to predict the actions of others, interpret subtle cues in communication, and better understand one's own behaviour. TOM is critical to understanding the pragmatic aspects of communication, and broader social behaviour.

The impaired TOM hypothesis predicts that those with an ASD are less able to infer their own and others mental states, and attempts to explain a number of social (pretend play, empathy) and communication (semantics and pragmatics) difficulties. Impaired TOM can also help explain the failure to recognize faux pas (unwritten social conventions) among those with autism (Baron-Cohen, 2008).

There are prominent experimental designs which have been used to measure TOM. Firstly, the Sally-Anne false-belief task uses a short comicstrip that requires participants to interpret a protagonist’s thought process. Specifically, this protagonist is misled, and will therefore search for an object in a location that they falsely believe it to be (see Figure 1.6 for a detailed description of this task). TD children are capable of succeeding at this task from around the age of four (Tager-Flusberg, 2007).
Figure 1.6: The Sally-Anne false-belief task, which assesses TOM skills. Sally has a basket, while her friend Anne has a box. Sally puts her marbles in the basket, and then leaves to another room. While she is absent, Anne takes the marbles from the basket and puts them in the box. When Sally returns, participants are asked where Sally will search for the marbles (i.e. she will search in the basket because she falsely believes they are still there; Tager-Flusberg, 2007).

The TOM hypothesis predicts that when engaged in the Sally-Anne false belief task, those with an ASD will have more difficulty inferring that the protagonist will search for the object in the wrong location. A large body of evidence supports this assertion (Baron-Cohen, Leslie & Frith, 1985; Mitchell & Lacohee, 1991; Mitchell, Saltmarsh & Russell, 1997), with a recent study identifying failure on the false belief task is independent of language deficits (Colle et al., 2007). Reed (1994) reported that only 4 of 22 (18%) ASD participants successfully completed the false belief task, by comparison to 21 (95%) TD participants. However, this study did not specify whether the sample also had intellectual deficits.

Secondly, the Social Attribution Task (SAT) tests TOM, and involves participants watching a short animation of basic visual stimuli (typically a large triangle, small triangle and small circle inside a rectangle) interacting. The
subject’s task is to identify a social narrative to the shapes, based upon the visual cues (Figure 1.7). The SAT requires participants to anthropomorphize social meaning out of ambiguous visual cues (Klin, 2000). In its first incarnation, Heider and Simmel (1944) found that with no cues, 33 of 34 normal participants described the animation in ‘human’ terms, with many describing specific animations in the exact same way.

![Figure 1.7: A still frame of the Social Attribution Task which assesses TOM skills. The three shapes form a cast of ‘characters’. Based upon their interactions participants are required to ‘anthropomorphize’ their movements into a social context. This is achieved by asking the participants follow up questions on what they thought happened, and what sort of ‘person’ each shape was. For instance, the ‘angry’ small circle may ‘aggressively’ bang on the rectangle, while the two ‘frightened’ triangles keep their distance. (Klin, 2000).](image)

The impaired TOM hypothesis predicts that ASD participants will be less able to anthropomorphize the shapes, and make less social and mental state attributions than TD participants. Klin (2000) compared HFA and TD participants on the SAT, and found the HFA group used significantly fewer TOM attributions cognitively (4.3% to 13.6%) and affectively (2.5% to 11.5%). Interestingly, HFA participants were more likely to invoke physical processes (such as ‘magnetic fields’) mitigating the relationship between the shapes.
Although the transcriber was blind to the purpose of this study, inter-rater reliability was not assessed. More research is required, as only a small number of studies have assessed TOM using the SAT.

A third design to test TOM abilities is the ‘strange stories’ test, which specifically tests social pragmatic abilities. Participants are presented with short stories where people say something they do not really mean. For example, a recipient of a birthday present may say ‘its lovely and just what I wanted’. This statement could be sincere as the present was what they wanted, or simply a means of sparing their friends feelings. These stories are phrased in such a way that the context makes the speakers motivation clear (Happè, 1994). Thus, the participant is required to specify the motivation of the comment in the story.

There is modest evidence that ASD participants have difficulty with this task. Jolliffe and Baron-Cohen (1999) found that those with an ASD performed significantly worse (84% correct) than TD participants (99%). Although a number of other studies support this finding (Gillberg, 1992; Rogers, Dziobek, Hassenstab, Wolf & Convit, 2007), in most cases ASD participants accurately assessed the speaker’s motivations (emphasized by 84% correctly identifying the speakers motivation in Joliffe and Baron-Cohen, 1999).

These findings provide evidence that an impaired TOM may underlie some of the social difficulties of autism, such as difficulties with empathy, relationships and pragmatic aspects of communication. Despite its success, a number of criticisms have been levelled at the impaired TOM hypothesis. Firstly, many individuals with an ASD demonstrate success on simple tests of TOM looking at others beliefs. Only when a task prompts complex emotions do they demonstrate impaired TOM (Baron-Cohen et al., 2005). Secondly, not all ASD
participants fail TOM tasks. It may be that those with autism undertake different
cognitive processes in their attempts to solve these tasks, which is an important
question to address. Thirdly, there has been some doubt cast upon whether tests
such as the false-belief task actually assess TOM (see Iao & Leekham, 2014).
Finally, and perhaps most importantly, the TOM hypothesis does not characterize
all symptoms, and does not explain any of the RRBS symptoms.

1.4.2 Systemizing theories

A number of theories have been proposed that elaborate upon the
predictions of the impaired TOM hypothesis. The first is a two-component theory
known as the empathizing-systemizing theory (E-S theory) (Baron-Cohen, 2009).
This theory suggests that humans develop two psychological dimensions of
behaviour; empathizing, the previously discussed drive to identify emotions in
others and respond to them appropriately, and systemizing, the drive to analyse
objects and events to understand there structure and predict their behaviour
(Baron-Cohen et al., 2005). These systems can be technical (such as a computer),
natural (such as evolution), motoric (such as a musical instrument) or abstract
(such as mathematics; Lawson, Baron-Cohen & Wheelwright, 2004).
Systemizing requires ‘if-then’ rules, and is best applied to systems that are 100%
lawful (such as a mathematical formula with a single degree of freedom; Baron-
Cohen, 2008).

Research on non-clinical samples has investigated whether there are
gender differences in empathizing and systemizing. In general, females are
stronger empathizers than males, whilst males are stronger systemizers than
females (Charlesworth & Dzur, 1987). Although small, these sex differences are
found within the first days of life with females staring longer at faces, while males stare longer at foreign objects. This is argued to reflect a biological disposition to empathizing and systemizing, which is further reinforced through development and socialization (Baron-Cohen, 2002).

Among ASD individuals, this gender discrepancy in empathizing and systemizing appears to be exaggerated (Baron-Cohen, 2009). Evidence from empathy and TOM studies suggest individuals with an ASD hypo-empathize, whilst their detailed focus on parts of objects and dependence on routine suggest that they hyper-systemize. This observation led Baron-Cohen and his colleagues to develop the Extreme Male Brain theory (EMB), which postulates that ASD may be characterized by an extreme variant of typically male cognitive abilities (Baron-Cohen, 2002). This theory helps explain the higher male prevalence of autism by suggesting that a male’s cognitive disposition makes them more vulnerable to ASD.

The EMB and E-S theories predict that those with an ASD will demonstrate reduced empathic abilities compared to TD males and females. Using the false-belief task, Happé (1995) found that more TD females (66%) passed the false-belief task than males (47.5%); consistent with the idea females are superior empathizers. Of interest, more TD males passed this task than ASD males, with only 20% of ASD participants successfully completing it.

A second prediction of the EMB and E-S theories is that ASD participants will demonstrate increased systemizing abilities compared to TD males. Baron-Cohen, Wheelwright, Spong, Scanhill and Lawson (2001) compared children with AS to TD children on a ‘folk physics’ test – which assessed everyday physics
problems such as the effect of a cog on a rotating wheel. This study found that those with AS performed significantly better than both TD males and females.

To date, only a very small body of research has directly tested the E-S and EMB theories. Moreover, no research has controlled for intelligence as a variable that may be moderating the results on empathizing and systemizing tests. It is reasonable to expect low functioning individuals with autism would not perform so well on systemizing tasks. The EMB and E-S theories require substantially more research to be assessed adequately, but provide a promising framework for understanding the cognitive profile of autism.

A more recent theory has attempted to explain why those with an ASD are stronger systemizers. This theory draws upon central coherence, which refers to the tendency of TD individuals to understand the global, ‘bigger picture’ meaning of objects or systems, at the expense of the detailed, local information. For example, when recalling a story, TD individuals will understand the ‘gist’ or overall picture (global processing) more so than fragments of conversation or specific details (local processing).

It has been suggested that those with an ASD have a weakened drive for central coherence (WCC theory), and focus their attention on details. Evidence for this theory has already been discussed in the context of RRBS through gestalt examples (Figure 1.5) (Bölte, et al., 2007). The WCC theories emphasis on local processing can begin to explain some of the sensory anomalies such as fragmented perception and hyper-sensitivity. For example, there is some evidence eidetic memory and absolute pitch is more prevalent in ASD (Heaton, Davis & Happè, 2008). However, this theory has been criticised as being a secondary outcome of E-S and EMB theories (Baron-Cohen, 2002; Happè & Frith, 2006).
The EMB, E-S and WCC theories extend upon the impaired TOM hypothesis by attempting to explain the routines, rituals and hypersensitivities prevalent in ASD. However, these theories possess a number of shortcomings. Firstly, they have focused upon psychometric descriptions of abilities, with little explanation provided on why ASD are characterized by hypo-empathizing and hyper-systemizing. The EMB theory postulates an extreme variant of male characteristics, but females are clearly not immutable to the disorder. Secondly, these theories do not account for several symptoms of ASD such as motor deficits and hypo-sensitivity.

Although estimates vary, TD children developed TOM and empathizing abilities between 18 months (Bultemann, Over, Carpenter & Tomasello, 2014) and four years of age in (Klin, 2000). An interesting developmental observation is that children with an ASD develop deficits in joint attention before this time. Joint attention begins to develop rapidly from six to 12 months in TD individuals (Hill, 2004A), with deficits appearing in ASD at approximately 10.4 months of age (Young, Brewer & Pattinson, 2003). These findings would appear to suggest that the origin of social deficits in ASD cannot be fully accounted for by TOM or hypo-empathizing, as deficits are present before these abilities develop.

1.4.3 Executive dysfunction theory

Another cognitive system believed to be impaired in ASD is executive functioning. Executive functioning is comprised of several overlapping mental systems such as planning, working memory, self-awareness, shifting and maintaining attention and inhibiting responses (Hill, 2004B). Following damage to the pre-frontal cortex, people demonstrate a need for sameness, rote repetition
of certain behaviours, difficulty switching attention and impulsivity (Rajendran & Mitchell, 2007). These deficits resemble a number of the social and RRBS symptoms of autism. This has led to the suggestion that these symptoms of autism are due to executive dysfunction (ED theory).

There are two major experimental designs to assess executive dysfunction. Firstly, the Tower of Hanoi Task (TOHT) requires placing varying sized discs on three rods (see Figure 1.8A for a detailed description). The TOHT requires thorough planning, and the ability to inhibit irrelevant responses (Russell & Jarrold, 1999). Secondly, the Wisconsin card sorting test (WCST) requires participants to sort cards into categories, whereby the sorting principle continually changes (see Figure 1.8B for a detailed description). The WCST measures maintenance skills, flexibility of thought and inhibition of an incorrect response.

![Figure 1.8: Two examples of tests of executive function. A. A simplified tower of Hanoi task (TOHT). The TOHT has three rods, and a number of varying sized discs that can be slid down these rods. On one of the rods, the discs need to be placed as a tower from largest to smallest. The subject has to recreate the tower on one of the other rods, without ever placing a larger disc on a smaller disc. B. The Wisconsin card sorting test (WCST). The WCST presents four ‘key cards’ (where their content varies in shape, colour and number) to a subject. The task of the participant is to sort a deck of cards into categories that match these four ‘key cards’. A participant is informed if they incorrectly categorize a card, but they are not given any clues as to what strategy to adopt. Once they categorize 10 cards correctly in succession, the sorting principle is changed and the participant has to adapt (Hill, 2004A).](image)
Ozonoff, Pennington and Rogers (1991) compared a group diagnosed with AD to TD participants on the TOHT and WCST. Using a scoring system based on participant’s efficiency to plan and solve the TOHT, the autistic group scored significantly fewer points by comparison to TD participants. On the WCST, the only significant difference found between the two groups was that those with autism tended to perseverate on a categorizing strategy more.

An advantage of executive functioning over the other cognitive theories is it has been linked to the development of joint attention. Dawson et al. (2002) compared children with ASD to developmentally delayed and TD children, to determine the relationship between executive functioning and joint attention. Participants completed tasks known to require ventral premotor cortex activity. The authors found that strong performance on tasks related to the ventromedial prefrontal cortex were significantly related to less joint attention impairment ($R=-20$). This research suggests that the skills involved in coordinating attention underpinned by executive function may contribute to joint attention.

ED theory provides another perspective on a number of autistic symptoms. Difficulty inhibiting incorrect responses on the TOHT and WCST may be linked to impulsivity observed in ASD. Moreover, the rigidity of autistic routines and rituals may stem from inflexibility of thought (a key characteristic of ED). Given the role of executive functioning in shifting attention, it is possible that ED may explain w-CC, as autistic participants have difficulty shifting between global and local processing (Happè & Frith, 2006). Indeed, some theorists suggest ED as a domain-general form of dysfunction that underlies the other cognitive theories (Iao & Leekham, 2014).
Nevertheless, a number of criticisms have been levelled at ED. Firstly, it cannot account for all autistic symptoms such as language abnormalities and sensory abnormalities. Secondly, it is unclear whether those with autism have a fundamental deficit in executive functioning, or another variable such as motivation leads to impaired performance on ED tasks (Hill, 2004A). For example, Ozonoff (1995) found that those with an ASD performed better at the WCST when it was completed on a computer by comparison to manually. This could be attributable to AS intolerance toward the verbal feedback they received in the manual task. Further, Mari, Castiello, Marks, Marrafa and Prior (2003) found that planning ability was related to IQ rather than autistic abilities.

In sum, the cognitive theories of autism reviewed in this thesis provide an incomplete explanation for different patterns of autistic symptoms. The impaired TOM hypothesis explains a number of social deficits such as empathy and communication deficits such as pragmatics, but fails to account for any of the RRBS. As a result, three similar theories emerged (E-S, EMB and WCC theory) that also explain a number of RRBS such as preoccupation with the parts of objects, rituals and routines and hyper-sensitive senses. However, none of these theories can explain the motor abnormalities of autism, or the development of joint attention problems. The ED theory accounts for a number of social deficits such as impulsivity and hyperactivity, and goes some way to explain the early onset of autistic symptoms. The literature on cognitive theories demonstrates the complexity of ASD, and emphasizes that the cognitive systems reviewed here likely overlap, and perhaps represent a common construct (Happè & Frith, 2006).
1.5 Conclusion

At its core, autism is characterized by insidious social deficits in joint attention, eye-gaze, imitation and empathy. More generalized social problems such as social anxiety and poor relationships may stem from these core deficits, or be independent problems in their own right. Language disturbance is particularly heterogeneous in severity, and most often involves failure to use context to communicate appropriately. The RRBS may in some way be a consequence of social deficits. Although sensory and motor deficits appear to be periphery symptoms, they can be just as disabling. The relationship between the triad of symptoms remains poorly understood and is a critical question for future research. Thus, ASD is characterized by a heterogeneous set of symptoms that are grouped together on a spectrum. Diagnosis is still made on the basis of these symptoms, with no reliable neurological or genetic marker identified.

Despite a long standing belief that society is facing an autism epidemic, the increase in prevalence is predominantly due to change in diagnostic criteria, increased awareness, and variation in sample size between studies. Autism is associated with a number of comorbid conditions including intellectual disability and Tourette’s. Thus, these comorbidities are important to disentangle when examining the presentation of individuals with ASD, and empirical findings.

A number of cognitive theories have been proposed to explain the symptoms of autism. However, due to its heterogeneous nature, no one theory has been able to provide a singular explanation. Impaired TOM-h explains a number of social symptoms, whilst the three systemizing theories (E-S theory, EMB theory and w-CC theory) begin to explain a number of RRBS symptoms. None of
these theories are able to explain the disturbed development of joint attention. However, the ED theory suggests cognitive development is impeded early in life with autism, and may indicate each of the theories reflect overlapping cognitive systems.
Chapter 2: Biology of Autism
2.1 Introduction

Autism is considered to be a heritable, neurodevelopmental disorder that is characterized by symptoms in three domains: social anomalies, verbal and non-verbal communication deficits and restricted and repetitive behaviours. The previous chapter illustrated a number of the major issues faced by researchers and clinicians to accurately define and diagnose this disorder. One such issue is that, to date, there are no reliable, biological markers. Thus, the goal of this chapter is to provide a review of genetic and neurobiological research into autism spectrum disorders (ASD).

From this research, a number of key arguments will be made. First, that ASD have a clear heritable component, but ambiguous genetic aetiology, where as-yet-unknown epigenetic factors contribute to altering the expression of the genome. Secondly, that autism has been associated with a broad range of neuroanatomical anomalies, which too frequently have not been replicated, or integrated together. Thirdly, that the abnormal brain connectivity model of autism provides a framework which goes some way to integrating many of the neurobiological findings in autism, but is presently hampered by a need for a priori specified networks to be tested. Finally, the recent evidence that the mirror neuron system may be abnormal in autism allows for the functional and structural properties of an a priori specified cortical network to be examined.
2.2 Genetic basis of Autism

2.2.1 Heritability

The prevalence of autism in the general population is approximately 0.05% to 1% (Baron-Cohen, 2002; Gillberg, Cederlund, Lamberg & Zeijlon, 2006). However, this prevalence is higher among those biologically related to an autistic case, ranging from 2% to 8% in siblings (Micali, Chakrabarti & Fombonne, 2004; Ormond & Seltzer, 2007; Vorstman et al., 2006). In relatives more distant than siblings the prevalence approaches the population level (Schellenberg et al., 2006). Moreover, Piven, Palmer, Jacobi, Childress and Arndt (1997) found that in 25 families with an autistic child, the fathers demonstrated significantly more social and restricted behaviour deficits, whilst the mother showed significantly more deficits in all three symptoms of the triad. The sibling recurrence-risk ratio ($\lambda_s$) is estimated to range from 50 to 150, meaning a sibling is 50 to 150 times more likely to have the disorder. Thus, there is strong evidence that the prevalence of autism increases as a function of relatedness to a diagnosed case.

Heritability has also been directly assessed in autism. Ritvo, Freeman, Mason, Mo and Ritvo (1985) found a 96% concordance rate for autism among monozygotic twins (MZ), by comparison to 24% for dizygotic (DZ) twins. Despite this research suggesting a clear genetic link, just how much variation in symptoms of ASD can be attributed to genes depends upon the breadth of the phenotype considered. Bailey et al. (1995) found a 60% concordance rate for monozygotic (MZ) twins compared to 0% for dizygotic (DZ) twins using a narrow phenotype of autism. However, when considering a broader phenotype,
91% concordance was found for MZ twins compared to 10% for DZ twins. These findings suggest genetics have a major role in autism, with some of the risk genes being inherited by other siblings.

More recent research has aimed to clarify the extent to which extreme autistic symptoms can be attributed to genetics or environmental factors using liability threshold modeling (LT) and DeFries-Fulker extreme analysis (DF). LT provides estimates of the degree to which a disease can be explained by genes ($h^2$) or the environment ($e^2$), whilst DF provides an estimate of the degree to which genes can account for the difference between probands and the population ($h^2_g$). Ronald et al. (2006) assessed 3,419 twin pairs from the general population aged between 7 and 9, and found autism to be highly heritable ($h^2=0.92$) and modestly due to non-shared environment ($e^2=0.08$). Genes were a relatively strong contributor to the difference in autistic symptoms between probands and the population ($h^2_g=0.66$). A graph of the correlation between MZ and DZ twins in this study is provided in Figure 2.1.

![Graph showing correlations of autistic symptoms between twins.](image)

**Figure 2.1:** Correlations of autistic symptoms between twins using the childhood Asperger syndrome test (CAST), looking at social impairments (SIs), communication impairments (CIs) and restricted, repetitive behaviours and interests (RRBIs). The twins investigated are monozygotic males (MZM), monozygotic females (MZF), dizygotic males (DZM), dizygotic females (DZF) and dizygotic opposite sex (DZOS; Ronald et al., 2006).
2.2.2 Chromosomal Anomalies

Although a genetic basis of autism is evident, the specific abnormalities have been difficult to identify. More than 10 genome wide studies have been conducted, such as the International Molecular Genetic Study of Autism Consortium (IMGSAC; 1998). These studies have found 17 of the 22 autosomal chromosomes and the X chromosome to have translocation sites, gene candidates and linkage peaks (Schellenberg et al., 2006; Wassink, Brzustowicz, Bartlett & Szatmari, 2004; see Figure 2.2). This suggests autism is not the result of a single defect being transmitted in a dominant, recessive or X-linked manner (Gupta & State, 2007).

Chromosomal defects in autism are usually studied with linkage analyses, which look within a family for co-occurrence of disease status with small segments of genetic variability (polymorphisms) throughout the chromosomes. These methods assume polymorphisms that are ‘distant’ from the disease will be random, whilst polymorphisms related to the disease phenotype will be transmitted in a non-random, linked manner due to a common inheritance (Kandel, Schwartz & Jessell, 2004; Wassink et al., 2004).

The most studied cytogenetic abnormality in autism is a defect at the 15q11-q13 loci. This is most commonly caused by Low Copy Repeat regions (LCR), which are recombinations of non-allelic genes located at different chromosomal regions, but derived from a common ancestral gene. LCR leads to defects including deletions, interstitial duplications, triplications and Supernumerary Marker Chromosomes (Kwasnicka-Crawford, Roberts and Scherer, 2007).
Figure 2.2: Depiction of cytogenetic regions of interest in autism across the entire human genome. Red and yellow bars correspond to de novo losses and gains observed in autistic cases. Green bars correspond to genes believed to modulate development of an ASD. Light green bars represent possible candidate genes, whilst purple shaded chromosomal regions correspond to high linkage peaks (Abrahams & Geschwind, 2008).
Maternally derived defects in this region confer a high risk for developing an ASD (>85%; Schanen, 2006). Although deletions and inversions have been associated with ASD (Muhle et al., 2004; Kwasnicka-Crawford et al., 2007), three maternal copies of the 15q11-q13 region is the most common defect (Gillberg, 1998). Nevertheless, chromosomal anomalies in the 15q11-q13-region only account for approximately 1 to 3% of autistic patients (Veenstra-VanderWeele & Cook, 2004). It is also possible maternal abnormalities in this region reflect comorbidity with Angelman Syndrome, intellectual disability, language delay, or motor ataxia (Muhle et al., 2004).

Several genome wide screens (i.e. IMGSAC, 1998) have identified a defect on chromosome 7q. Maternal imprinting, inversion and translocation defects on the long arm of chromosome 7q have been found to contribute to development of an ASD (Molloy, Keddache & Martin, 2005; Veenstra-VanderWeele & Cook, 2004). Linkage studies have implicated a region in the vicinity of 7q to speech and language disorder, which may suggest 7q anomalies contribute to communication impairments in autism (Vincent et al., 2000). Ashley-Koch et al. (1999) investigated a family of seven individuals (two grandparents, their daughter and her husband, and their three children [one male, and a male/female DZ pair]). All three children and their mother possessed an inversion (a breakage and rearrangement of a chromosome) on the long arm of chromosome 7. Only the male siblings met the criteria for autism. This male only expression of autism may suggest that a defect at 7q may be necessary but not sufficient for developing autism. Linkage results revealed five markers to be
significantly associated to autism (D7S495, D7S1824, D7S684, D7S640 and D7S2527; see Figure 2.3).

Figure 2.3: Diagram of Chromosome 7 for multiplex family possessing inversion on the long arm. The small, crossed bar on the left of the chromosome represents the inversion location in this family. The small dotted bar denotes areas associated with speech and language impairment. The remaining bars are areas of defect found in past studies. FRA7E and FRA7G are ‘fragile sites’, believed to be involved in chromosomal rearrangements, and likely to be involved in the inversion present in this family (Ashley-Koch et al., 1999).

Although less research has been conducted, anomalies on chromosome 2q have also been linked to autism. A genome wide study conducted by the IMGSAC (2001) found that region 2q21-33 had the strongest association to autism of any chromosomal defect. A recent meta-analysis of linkage studies found region 2q37 to be the most pronounced defect on chromosome 2 (Vortsman et al., 2006). However, Gallagher et al. (2003) report an unclear interstitial deletion of either 2q32.1 or 2q32.3 (see Figure 2.4). A broader study by Casas et al. (2004) investigated the phenotype associated with 2q in 66 children. In this sample, 59 out of 66 (89%) had developmental delay, whilst 16 out of 66 (24%) presented with autistic behaviour, making it one of the strongest
candidate regions for autism. Furthermore, 30 out of 66 (45%) experienced hypotonia, a symptom often expressed as a muscular abnormality in autism.

![Image](normal_chr_2_del_2.png)

**Figure 2.5:** Small interstitial deletion on chromosome 2 in a young male with high functioning autism. The left pictures depict two homologues with the normal binding patterns, whilst the right pictures depict a deletion of 2q32.1 and 2q32.3.

Research has found chromosome 17 possesses disturbance related to ASD on the short arm. A recent meta-analysis of eight genome wide scans report that when applying a maximum odds ratio of 1.5 (i.e. strict significance thresholds), 17p11-17q11 was the only region that reached significance in more than one of these studies (IMGSAC, 2001). However, differences in ethnicity, diagnostic criteria and methodology between the studies are likely confounds.

Abnormalities related to autism have been found on the long arm of chromosome 22, around 22q11 or 22q13, have been related to dysmorphic facial features, developmental delay, hypotonia and expressive language and speech delay (Freitag, 2007). Case reports have identified abnormalities in chromosome 22 including *de novo* deletions (Manning et al., 2004) and duplications
(Assumpcao, 1998). However, other research has found no evidence for deficits on chromosome 22 in individuals with an ASD (Ogilvie, Moore, Daker, Palferman & Docherty, 2000).

There are a number of limitations to studying chromosomal defects in autism. Firstly, when considering the literature as a whole, cytogenetic defects account for approximately 3 to 5% of autistic cases (Freitag, 2007). Thus, the majority of variance in autism is not accounted for by cytogenetic methods. Recent advances in array based approaches (such as increased resolution) may reveal that rare structural variants in chromosomes account for a larger amount of variance (Abrahams & Geschwind, 2008). Secondly, the linkage signals found in autism are weak by Mendelian standards, with replication being the exception to the rule. Thirdly, the availability of probes may influence the results. Certain chromosomal regions may be over represented due to availability of probes; whereas other regions may be under represented due to lack of availability of probes. One means of overcoming this limitation is to look at gene candidates within a chromosome. These methods possess a higher resolution, allowing genes of weaker effect to be detected (Veenstra-VanderWheele & Cook, 2004).

2.2.3 Gene Candidates

Studies investigating specific gene candidates for autism have typically used association methods. This method looks for Single Nucleotide Polymorphisms (SNP), which are sequence variations in DNA occurring within one nucleotide. Mounting evidence suggests autism is oligogenic, meaning the disease phenotype is the result of multiple, interacting genes (Bespalova & Buxbaum, 2003). Currently, it is unknown how genes combine and interact to
produce the autism phenotype. One approach considers autism to be a *locus* *heterogeneous* disorder, meaning that defects in different genes may cause the same phenotype. Another approach considers autism to be an *epistatic* disorder, where multiple genes influence the expression of another gene (Veenstra-VanderWeele & Cook, 2004). Although it is believed that 15 to 100 genes are implicated in the autism phenotype, this section will discuss those genes with the strongest evidence for association with ASD.

Genes in the Gamma-Aminobutyric Acid (GABA) system have been linked to autism. GABA is the major transmitter present in a number of inhibitory interneurons, such as basket and Purkinje cells in the cerebellum (Kandel et al., 2000). Although GABA receptor genes are located throughout the human genome, fine mapping has narrowed the autism susceptibility gene to *GABRB3*, located at the 15q11-13 locus. *GABRB3* is a gene that encodes a protein that serves as a receptor for GABA in the central nervous system (Ma et al., 2005).

Several markers on this gene have been associated with developing an ASD (Curran et al., 2006). Buxbaum et al. (2002) investigated 80 autism families, and found that marker 155CA-2 on *GABRB3* was significantly associated with autism. Other GABA genes have been linked to the disorder. Ma et al. (2005) found significant associations at *GABRA4* and *GABRA2*, both located at chromosome 4p12 (Ma et al., 2005). Limited research has explored the possibility that *GABRB3* anomalies are linked to repetitive and restrictive behaviours in those with an ASD (Shao et al., 2003), but more research is necessary. Overall, despite a number of studies identifying defects in GABA receptor genes, the findings have been inconsistent (Veenstra-VanderWeele & Cook, 2004).
Several genes at chromosome 7q have been implicated in autistic symptoms. Firstly, ‘Forkhead Box P2’ (FOXP2) is believed to have a role in the development of Broca’s area. This gene differs by only two amino acids in humans by comparison to chimpanzees, and has been implicated as a possible gene in the evolution of speech and language (Enard et al., 2002), and language disorder (Lai, Fisher, Hurst, Vargha-Khadem & Monaco, 2001). The IMGAC (1998) found limited evidence for a FOXP2 defect at chromosome 7q32.1-34 in autism, which may be an important step in understanding the genetic etiology of communication deficits. However, the role of FOXP2 is disputed, and the majority of studies have found no evidence for a defect in this gene in autism (Bauman & Kemper, 2005). Given that language deficits have been found in chromosomal linkage studies at 7q (Vincent et al., 2000), the contribution of FOXP2 is important to clarify.

Secondly, Reelin (RELN; located at 7q22) is a gene that codes for glycoprotein with an important role in neural migration and connectivity. One study reports RELN defects at marker D7S495 (Skaar et al., 2005); the same peak region as the previously discussed 7q cytogenetic study (see Figure 2.3). Persico et al. (2001) compared the RELN gene of those with an ASD (N=95) to TD participants (N=186), and found those with an ASD had significantly more ‘long’ (≥ 11 repeats) GGC repeats (17.9% compared to 9.1%) on the RELN gene. A trinucleotide repeat at 7q22 is the most common RELN defect found in autism (Freitag, 2007; Persico et al., 2001).

Although the relevance of RELN to developing an ASD is unclear, recent research suggests anomalies here may contribute to abnormal Purkinje cell volumes in the cerebellum (Fatemi, Stary, Halt & Realmuto, 2001).
Unfortunately this study did not genotype these individuals to determine whether defects were present in the RELN gene. Moreover, another gene responsible for secreting proteins (LAMB1; located at 7q31) is also responsible for cell migration to this area, and may influence the neural development of autism (Persico & Bourgeron, 2006).

Thirdly, the gene *engrailed 2* (EN2) also has an important role in the development of the cerebellum, and is located at 7q36.3. Interest in this gene came from mice studies, which demonstrated that defects in EN2 led to similar cerebellar abnormalities to those with autism (Kuemerle, Gulden, Cherosky, Williams & Herrup, 2007). Gharani, Benayed, Mancuso, Brzustowicz and Millonig (2004) investigated 60 families, of which at least two members had an ASD associated with EN2 transmission abnormalities. They found two alleles were over-transmitted in affected siblings; rs1867972 (65%) and rs1867973 (68%). A further haplotype analysis of these two alleles determined the A-C haplotype had an observed frequency of 68.8% in affected individuals. In mice, prolonged expression of EN2 in Purkinje cells leads to reduced numbers of this cell; a deficit observed in autism. Consequently, it is important that future research corroborates genetic and anatomical studies in humans that have identified over-transmission of EN2, and reduced Purkinje cell numbers.

The serotonin transporter gene (*SLC6A4*; located at chromosome 17q11.1–q12) has been associated with autism. *SLC6A4* modulates levels of extracellular and synaptic serotonin in the brain (Wassink et al., 2004). These receptors are predominantly found in the dorsal midbrain and brainstem, which are known regions of neurological disturbance among individuals with an ASD (Bauman & Kemper, 2004). Interest in this gene came from a number of studies
that found elevated serotonin levels (hyperserotonemia) in platelets among those with autism (Anderson, Horne, Chatterjee & Cohen, 1990).

Evidence for \textit{SLC6A4} anomalies among those with an ASD is mixed. Devlin et al. (2005) investigated \textit{SLC6A4} in a family based sample ($N=390$ families, 1528 individuals), and found that the short allele of HTTLPR was the only locus of \textit{SLC6A4} that was over transmitted in those with autism. However studies have found over transmission of the long allele (Klauck et al., 1997) and no association at all between \textit{SLC6A4} and autism (Persico et al., 2000). A study of 71 children and their families by Tordjman et al. (2001) has linked \textit{SLC6A4} to social deficits in individuals with an ASD. This research found that the \textit{s} (small) allele was transmitted at higher rates in those with severe social and communicative deficits, whilst the \textit{l} (long) allele was transmitted at higher rates among those with moderate to mild deficits in these domains. A medium effect was found for this association (Cramers’ statistic $\Phi^2$ ranges from 0.08 to 0.18), but more research is necessary.

Because ASD afflicts more males than females (male to female ratio approximately 4:1 for autism and 8:1 for AS) (Fombonne et al., 2005), research has investigated gene candidates on the sex chromosomes. Two neuroligin genes have been implicated in autism (\textit{NLGN3} and \textit{NLGN4}), located at Xq13 and Xp22.3 respectively (Jamain et al., 2003). In a family spanning five generations, Laumonnier et al. (2004) found a two base-pair deletion at \textit{NLGN4} in 12 male family members affected with X-linked mental retardation with or without autism, which suggests deletion of this gene may pre-dispose to an abnormal development. Jamain et al. (2003) however identified a frameshift mutation in two boys with ASD in the same family, who did not possess mental retardation.
Despite these findings, other research has found no link between NLGN4 and ASD (Vincent et al., 2004). This body of research indicates that neuroligins may play a role in the development of PDD, but are not core antecedents to autism.

In sum, the contribution of specific genes to the autism phenotype is not well understood. Candidate gene studies have been conducted on the basis of relatively little knowledge of pharmacology, developmental neuropathology and chromosomal abnormalities that occur in ASD (Veesntra-VanderWeele & Cook, 2004). Replication of genetic defects has not reliably been achieved, and no single genetic abnormality has been found to account for more than 1 to 2% of autistic cases (Abrahams & Geschwind, 2008). Moreover genes such as GABRB3 and NLGN4 have been linked to other disorders. Their linkage to the autism phenotype may be mistaken for comorbid disorders such as Angelman Syndrome and Prader-Willi Syndrome. A number of genes on chromosome 7q (RELN and EN2) are promising due to their role in cerebellar development, in addition to SLCA64, which has a role in the development of the limbic system. However, it is important to recognize that the technologies for linkage and association analyses are progressing rapidly, allowing for higher resolution techniques. This means that genes of weaker effect, which are more reliably linked to autism, may yet to be detected.

2.2.4 Teratogens and Vaccines

Pre and peri-natal teratogens such as viruses, toxins, dietary factors, oxidative stress, neuroinflammation, and mitochondrial dysfunction have been implicated in ASD (Amaral, 2011). Although its been suggested that ASD is associated with a higher level of toxins and viruses in utero, specific pre-natal
abnormalities have not been reliably replicated (Taylor, 2006). Although it is unlikely pre-natal exposure to toxins directly causes autism, it remains possible that toxins can disturb a genetic liability to produce the autism phenotype (Goodman, 1990).

The strongest teratogens linked to autism are congenital diseases during pregnancy, including rubella and Cytomegalovirus (CMV) (Persico & Bourgeron, 2006). CMV is a DNA based virus belonging to the herpesviridae family, and can be experienced during pregnancy or early infancy. Evidence for a role of CMV in autism etiology is limited. Yamashita, Fujimoto, Nakajima, Isagai and Matsuishi (2003) investigated 7 children with CMV, of which 2 went on to receive a diagnosis of autism. However, this study failed to report sampling methods, along with developmental information related to the course of the pregnancy or family history of autism. Moreover, the authors fail to provide any theoretical link between CMV and specific brain abnormalities. Although one other study has made a similar report (Markowitz, 1983), with limited evidence it is difficult to determine how CMV and autism are related.

Another teratogen linked to developing an ASD is thalidomide; a sedative drug once used for morning sickness during pregnancy. Thalidomide has similar effects to valproic acid, another teratogen linked to autism. Miller, et al. (2005) reviewed five studies investigating embryogenic deficits, including one, which looked at pre-natal thalidomide exposure ($N=86$). Of this sample, 5% had a diagnosis of autism. It is important to recognize that autism was one of 14 other problems attributed to thalidomide including mental retardation, cardiovascular disease and cranial nerve damage. This appears to suggest that pre-natal exposure to thalidomide can cause a diverse range of problems, which depends upon how
much thalidomide the developing child was exposed to, and the period of pre-
natal development at the time of exposure. It is believed that 20-24 days post
conception is a critical period for increased autism risk (Arndt, Stodgell &
Rodier, 2005). Thus although thalidomide can lead to autistic symptoms, it
appears to be a catalyst for general brain damage, which can exacerbate a
susceptibility at a critical period.

A more recent hypothesis has been that certain vaccines administered to
children may trigger an unknown genetic susceptibility and cause autism.
Wakefield et al. (1998) published a controversial paper linking the Measles,
Mumps and Rubella vaccination (MMR) to autism. This paper reported 12
children with chronic enterocolitis (bowel disease) who also developed
behavioural problems. Of this sample, 8 children were reported to have
developed behavioural symptoms within six days of their MMR. One parental
report was provided which indicated the behavioural symptoms included
disinterest in siblings and lack of play.

There are a large number of problems with the methodology and
conclusions of this study. Firstly, it did not utilize a control group of children
who received the MMR vaccine but did not have chronic enterocolitis. With no
baseline group to compare to, a sample of children with bowel disease is highly
biased. It also emerged later that most of the sample were litigants against MMR
vaccine manufacturers, and the research was funded by legal aid (Taylor, 2006).
Secondly, no epidemiological, biological or virological evidence was provided to
link the MMR vaccine to autism. At best, this study has established that the onset
of behavioural symptoms has co-occurred within a similar time frame to
vaccination.
Thirdly, the symptoms of the children described are vague, with no detail of what criteria was used to diagnose autism, or whether they had received a formal diagnosis. Behavioural problems were noted by parents and the child’s physician and were not adequately described. Currently, the Centers for Disease Control and Prevention and the Institute of Medicine have concluded that no reliable evidence links MMR to autism (Taylor, 2006). Population studies have found no link between autism and MMR (Gillberg & Heijbel, 1998), while no association has been made between bowel problems and autism (Fombonne & Chakrabarti, 2001).

The mercury preservative in vaccine (thiomersal) has also been claimed to prime autism. Trace amounts of thiomersal are used in a number of vaccines to ensure sterility. Geier and Geier (2003) used the Vaccine Adverse Events Reporting System (VAERS - a database of all adverse reactions to vaccines in the USA) to look at the association between autism and thiomersal. They compared the incidence of autism following Diptheria-Tetanus-acellular Petussis (DTAP) vaccines with thiomersal, against DTAP vaccines without thiomersal. A statistically significant increase in the incidence of autism with thiomersal containing DTAP was reported ($R^2=0.98$). A pivotal limitation to this study is that the VAERS is a passive reporting system, to which anyone can report an adverse effect following a vaccination. Consequently, the reports of autism were not mandated, meaning diagnosis was not validated (Parker, Schwartz, Todd & Pickering, 2004). Moreover the mean age of children in this study was 1.7 years of age, which is below the age that a reliable diagnosis of autism can be made. These limitations suggest it is not clear whether the reported adverse events
qualify as autism. Moreover, in a review of 12 studies, Parker et al. (2004) reported no evidence of an association between autism and thiomersal.

An important question to address is why the reports of an association between vaccines and autism have propagated despite limited evidence. Firstly, the prevalence of autism has increased conjointly with more vaccines, which has led some authors to make an association. The increased prevalence of autism is now attributed to heightened awareness of the disorder, more specific diagnostic criteria, and larger epidemiology studies leading to more accurate prevalence estimates (Wing & Potter, 2002). Moreover, the prevalence of autism has been contrasted between time periods where MMR was administered, and periods where it has not. For instance, in Yokohama, the MMR program was terminated in 1993. Thus, Honda, Shimizu and Rutter (2005) were able to look at the prevalence of autism during a period of MMR, then following the termination of MMR. Their results indicated that despite vaccinations decreasing from 42.9% to 1.8% from 1990 to 1993, the incidence of ASD continued to rise among non-vaccinated children.

Secondly, parental testimonies identify the onset of autism shortly after their children are vaccinated. Developmental research indicates that many autistic symptoms emerge around this time. Moreover, research has identified that parents’ recall of their child’s development can be biased. A committee on safety of medicines (1999) investigated the records of children with autism who had parents that identified MMR as the trigger of the disorder. In 39% of these children, there was evidence that developmental concerns existed prior to the vaccination. However, only 1% of parents in this sample recalled this earlier concern. Overall, there is no reliable evidence that vaccination is a fundamental
cause of autism. The evidence suggests that in rare cases vaccinations may trigger autistic-like symptoms from a genetic liability.

2.2.5 Epigenetic Contributions

Epigenetics (literally meaning on, or above genes) refers to any genetic modification other than changes in the actual DNA sequence. There are two examples of epigenetic modification studied among those with an ASD. Firstly, genomic imprinting regulates genes via epigenetic modification that leads to parent-specific gene expression. In some imprinted genes, the cell will only use the maternal gene copy to make proteins, whilst in others it will only use the paternal copy.

At present, only a small body of research has been completed on imprinting defects in autism. Maternally derived imprinting defects (particularly duplications) have been found to confer higher risk to autism than paternal defects, particularly in the genes UBE3A and ATP10A at 15q11-q13 (Schanen, 2006). In a study of post-mortem brains in autism, Jiang et al. (2004) found abnormal DNA methylation at UBE3A in one of these brains. Protein E6-AP was found to be abnormal in 3 of 17 Cerebellum’s, and 4 of 17 Cerebral Cortices. It is important to recognize that UBE3A is the gene responsible for Angelman Syndrome. Similarly to the findings with GABRB3, this finding may reflect comorbidity between autism and AMS. At present, little research has investigated ATP10A with one study reporting linkage disequilibrium at this gene (Nurmi et al., 2001). More research is necessary on possible imprinting defects at cytogenetic regions of interest (2q, 7q, 17q).
Secondly, *Copy Number Variation* (CNV) refers to differences in the number of copies of a particular gene from the genome of one individual to another. *De novo* CNV occurs when the number of copies in a particular gene spontaneously changes through no mode of inheritance or transmission. *De novo* CNV may be a random mutation, or due to unknown environmental factors. Sebat et al. (2007) recently investigated *de novo* CNV in those with autism with no family history, those with autism from a multiplex family, and a typically developing (TD) sample. The frequency of CNV was 10% (12 out of 118) for autistic individuals with no family history, 2% (2 out of 77) for those with autism from multiplex families, and 1% (2 out of 196) for TD participants. It is possible that the limited resolution of genome microarray scans may not be able to reveal yet undetected CNV leading to autism susceptibility. Zhao et al. (2007) speculate that *de novo* CNV may be responsible for upward of 30% of autism cases.

The specific epigenetic factors that confer risk to autism remain a question for future research. Skuse et al. (1997) suggests that an imprinted locus on the maternal X-chromosome confers increased risk of epigenetic mutations in males, as they don’t have a paternal X chromosome. A similar theory outlined by Zhao et al. (2007) suggest *de novo* CNV have higher penetrance in males along the maternal germline, where unknown factors buffer females from autism. These theories speculate that mutations linked to autism could occur in upwards of 100s of genes. Studies on mice have identified a number of gene candidates on the maternal X chromosome (*Xl3b, Xl4b* and *Xl4c*; Marco & Skuse, 2006), but ortholog genes have not been found in humans (Schanen, 2006).
2.2.6 Conclusion

Overall, genetic research into ASD has not produced definitive findings. If autism is considered as a single disorder, it does not fit known inheritance patterns (Miles, 2011), with cytogenetic and gene candidate studies accounting for less than 5% of cases. Recent advances in epigenetics are starting to build evidence that unknown factors may be responsible for altering the expression of the genome in those with autism. Although research has investigated the role of viral agents and vaccines, the evidence suggests these factors are unlikely to be responsible for autism. Identification of any specific agents that can lead to de novo CNV or act upon a genetic susceptibility is an important question that needs to be addressed. Moreover, biological systems approaches may be valuable to explain the genetic heterogeneity of the disorder. For instance, given that GABRB3 has been linked to development of ASD, it may be valuable to investigate the role of other genes in the GABA system (such as DLX5 located at chromosome 7).

2.3 Neurophysiological Links to Autism

Similarly to the genetics literature, neurological abnormalities in those with ASD are heterogeneous, with a reliable neuromarker yet to be identified. When examining and interpreting anatomical findings, it is important to recognize the developmental nature of the disorder. Brain abnormalities associated with autism are not necessarily static, and can change throughout the lifespan. Moreover, where a particular brain region is impaired, it may not be clear if this is attributable to localised disturbance in that region, abnormal
interactions with other brain regions, or a combination of both (Bauman & Kemper, 2004). This section will review brain regions that are most commonly found to be pathological in autism, and some of the key differences in neurodevelopment that occur in autism compared to TD participants.

2.3.1 The Cerebellum and Brain Stem

The cerebellum is one of the most commonly disturbed brain regions in autism. A number of the strongest candidate genes in autism (RELN and EN2) are responsible for cell migration, connectivity and patterning in the cerebellum (Courchesne, 2004; Hashimoto et al., 1995). Although cerebellar pathology in autism is heterogenous, Courchesne (1995) reviewed 16 studies of 240 autistic brains using Magnetic Resonance Imaging (MRI) and autopsy, and found the most common cerebellar deficit observed was hypoplasia (under developed tissue). However, more recent studies have identified a larger cerebellum size in participants with ASD (Courchesne et al., 2001; Hardan, Minshew, Harenski & Keshavan., 2001; Sparks et al., 2002), which may suggest individuals with an ASD demonstrate greater variability in cerebellum size.

Purkinje cells are one of the principal cortical outputs of the cerebellum, and selective abnormalities in these cells have been associated with ASD (Kern, 2003). In a review of 24 post-mortem autistic cases, Amaral Schumann and Nordahl (2008) identified 19 (79%) studies to have reported decreased density in Purkinje cells. The most severe loss of Purkinje cells in those with an ASD has been found to occur at posterior vermian lobules VI-VII and VIII-X, with one microscopic analysis revealing 50-60% reduced numbers in this area (Courchesne et al., 1994).
Purkinje cells have also been found to be smaller in size among those with an ASD. Fatemi et al. (2002) Nissl-stained a 14-micron thick coronal section of the cerebellum in post-mortem autistic and TD participants, and found that those with autism had on average 50% smaller Purkinje cells (661.18 $\mu m^2$, compared to 502.31 $\mu m^2$). Although it remains unknown why Purkinje cells are vulnerable in autism, a clue may be there link to common autism genes GABRB3 and RELN (Fatemi et al., 2009). It also needs to be acknowledged that other abnormalities in the cerebellum have been linked to autism, such as deficits in the fastigial and globose nucleus (Bauman & Kemper, 2005).

Very little research to date has examined the consequences that an abnormal cerebellum would have on participants with an ASD. Using functional magnetic resonance imaging (fMRI), Allen, Müller and Courchesne (2004) compared a group of autistic adults to TD participants, and reported increased activation in the cerebellum during motor tasks, but reduced activation during visual attention tasks (a finding also made by Allen & Courchesne, 2003). Furthermore, Harris, Courchesne, Townsend, Carper and Lord (1999) compared those with autism to TD participants on an attention based task, and found that errors in orientating information correctly were significantly associated with smaller cerebellar vermian lobules VI and VII ($R=-.60$). Thus, cerebellar deficits in autism may impede upon basic information processing.

As the brainstem is a hindbrain structure closely linked to the cerebellum, it has also been investigated in participants with an ASD. However, only a small amount of systematic research has been conducted on this region. Using MRI, Hashimoto et al. (1995) compared 102 autistic participants to 112 TD participants, and found the overall size of the brainstem to be significantly
smaller in the autistic group. Kemper and Bauman (1993) and Bailey et al. (1998) provide further support for this finding.

Evidence for lesions in specific brainstem regions is limited. Rodier, Ingram, Tisdale, Nelson and Romano (1996) reported an absence of neurons in the superior olivary nucleus (a part of the pons believed to have a role in auditory stimuli), and fewer neurons in the facial motor nuclei. However, these findings were based upon a single post-mortem case, and do not appear to have been replicated. It remains unclear if brainstem defects are an epiphenomenon of damaged connections to the cerebellum.

2.3.2 The Frontal Lobe

Damage to the pre-frontal cortex leads to problems with executive functioning in TD participants (Rajendran & Mitchell, 2007). ASD participants often demonstrate impaired executive function (Hill, 2004A; Hill, 2004B), which has prompted research on frontal lobe anomalies. Among those with autism, it has been argued that cytoarchitectonic organization of the prefrontal cortex is disturbed (Courchesne & Pierce, 2005). Casanova et al. (2006) compared 6 post-mortem brains with autism to 6 post-mortem TD brains, and found that minicolumnar width was reduced by 1.46 μm (or 5.54%) in autism. Separate minicolumns were packed closer together in autism, but the total number of cells per column was normal. Although similar deficits have been found in other studies (Buxhoeveden et al., 2006; Buxhoeveden, Fobbs & Casanova, 2002), Amaral et al. (2008) points out that only 14 post-mortem brains in the literature have been studied for minicolumnar deficits, of which 10 had mental retardation, and 9 seizures, thus confounding the link to autism specifically.
2.3.3 The Amygdala and Fusiform Gyrus

The amygdala, along with the orbito-frontal gyrus and superior temporal gyrus, is believed to have a role in social intelligence (Baron-Cohen, Ring, Bullmore, Wheelwright, Ashwin & Williams, 2000). As a result, the amygdala has been postulated to be an important structure for theory of mind abilities, and a neural locus for social difficulties in autism. In children with autism, there is evidence that the amygdala is larger (Giedd, 1997). In a study comparing 3 and 4 year old children with autism to developmentally delayed and TD children, Sparks et al. (2002) identified the amygdala was between 13-16% larger in those with autism. Schumann et al. (2004) made a similar finding in participants with an ASD (>15% larger). In adult samples, a smaller amygdala has been observed (Pierce & Courchesne, 2001), with a post-mortem study of individuals with ASD who were aged between 10-44 years of age revealing significantly fewer neurons in the amygdala (Schumann & Amaral, 2006).

It has been theorized that the right amygdala in particular has an important role in threat detection and evaluating fear (Amaral & Corbett, 2002). Thus, deficits in the right amygdala may be linked to social anxiety in autism. Juranek, Filipek, Berenji, Modahl, Osann and Spence (2006) found an enlarged right amygdala volume explained 22% of variance in anxiety and depression levels, where differences in the left amygdala did not reach significance.

On the other hand, the left amygdala has been linked to deficits recognizing emotions in faces. Using MRI, Dalton et al. (2005) found that AS participants were less able to recognize emotions in faces (98.5% correct compared to 85%), which was associated with significantly greater activation in the left amygdala and the orbitofrontal gyrus; a result previously established by
Baron-Cohen et al. (2000). More recent evidence found no volumetric differences in the amygdala between TD and ASD participants aged between 8 and 12, but identified changes in right amygdala volume were linked to appropriate eye contact (Barnea-Goraly et al., 2014). The functional role of the amygdala in autism remains an on-going question.

Another brain region with a role in face processing is the fusiform gyrus; a small ridge of cells on the temporal lobes, ventral to the limbic system. It has been suggested that abnormalities in this region may contribute to problems interpreting the emotional content of faces. van-Kooten et al. (2008) conducted post-mortem research on 7 brains with autism, and 10 TD brains. Overall the autism brains demonstrated reduced neuron density in layer III (-13.1%), and reduced neuron numbers in layers III (-23.7%), V (-14.3%) and VI (-10.6%). Functional differences in the fusiform gyrus have also been reported, with Critchley et al. (2000) identifying hyperactivity in this region in participants with an ASD in response to faces. Nevertheless, not all research has supported fusiform gyrus disturbance in autism (Hadjikhani et al., 2004).

2.3.4 The Hippocampus

The hippocampus has been linked to autism, but demonstrated inconsistent findings, with its possible role not well understood. Saitoh, Karns and Courchesne (2001) investigated autistic participants with an age range of 29 months to 42 years to look at longitudinal changes in hippocampus development. These changes were compared to age-matched TD participants. Their findings are summarized in Figure 2.5, with a small hippocampal region known as area dentata (involved in the formation of memory circuits) being significantly smaller.
in the autism group at all ages by comparison to TD participants. The biggest difference was observed in the group aged between 2 and 4 years, where this region was 13.5% smaller in the autism group.

![Graph](image_url)

**Figure 2.5:** Size in millimeters squared of AD (area dentata) in autistic individuals and TDs across five age groups (2 to 4, 5 to 6, 7 to 12, 12 to 19 and 20 to 43 years of age). At all ages, the area dentata was smaller in the autistic sample. (Saitoh et al., 2001).

However, there are a number of limitations to this study. Firstly, sample size varies between age groups. The 7 to 12 and 13 to 19 age groups had more TD participants, whilst the remaining groups had more autistic participants. Differences in the sample size are likely to have influenced the variance between the two groups. Secondly, the hippocampus has been implicated as having a role in the development of temporal lobe epilepsy (Kandel et al., 2000, page 931). In this sample, 25.4% of autistic participants had a history of seizures. Abnormalities of the hippocampus observed may reflect comorbidity with epilepsy. Other research has found no difference in the size or activity of the hippocampus between autistic and TD populations (i.e. Piven et al., 1997), which may indicate abnormalities in the hippocampus reflect a comorbidity with epilepsy.
2.3.5 The Basal Ganglia

A small amount of research has implicated the basal ganglia in autism pathology. The basal ganglia possesses four interconnected nuclei, and is believed to have a role in voluntary movement. As such, it has been implicated in motor disturbance in autism. Sears, Vest, Mohamed, Bailey, Ranson and Piven (1999) used MRI to measure the size of the basal ganglia in a sample with High Functioning Autism (HFA) and TD participants. They found that the caudate, but not the putamen or globus pallidus, was significantly larger in the autism group (8.69cm³) compared to the TD group (8.03cm³). This increased caudate size explained a small amount of variance in restricted abilities and repetitive behaviours ($R^2=.13$). Previous research has made a similar finding (Jacobson, Couteur, Howlin & Rutter, 1988), whilst metabolic deficits (Horwitz, Rumsey, Grady, Rapoport, 1988) have also been identified in the basal ganglia of participants with an ASD.

A number of studies have also reported no difference in the basal ganglia between ASD and TD participants. Hardan, Kilpatrick, Keshavan and Minshew (2003) age and gender-matched HFA and TD participants, and report no significant difference between the groups in any region of the basal ganglia. Similarly, Creasey, Rumsey, Schwartz, Duara, Rapoport and Rapoport (1986) reported no abnormalities in the caudate nuclei of individuals with autism. These discrepant findings are likely attributable to the heterogenous nature of motor abnormalities in autism. Furthermore, voluntary movement also involves goal-directed decision making in the premotor cortex, and execution by motor neurons in the brain stem and spinal cord. Thus, motor deficits could be present in autism, in the absence of basal ganglia disturbance.
2.3.6 Brain Development

As the above research shows, a wide range of neuroanatomical disturbances occur in autism spanning many regions of the brain. In the cerebellum alone, deficits include hyper-activity (Allen et al., 2004), reduced cell size (Fatemi et al., 2002), and increased (Courchesne et al., 2001) and decreased (Hashimoto et al., 1995) cortical volume. However, there is emerging evidence that the nature of brain disturbances in ASD vary, depending on the period of development. Thus, all neuropathological findings in autism need to be understood within the context of the participant’s age and developmental window. Thus, this next section will consider both cross-sectional and longitudinal research that has investigated age-related brain changes in autism.

Abnormal brain volume is one of the most common observations in autism. Kanner (1943), in his first description of the disorder, noted that five of 11 autistic children had an enlarged head. In a later study of head size with a broad age-range between 2-16 years, Fombonne et al. (1999) identified macrocephaly (head circumference > 97th percentile) in 16.7% participants with autism; a level much higher than the expected 3% in TD individuals. However, microcephaly (head circumference < 3rd percentile) was also identified in 15.1% of the sample. Although this study did not specifically link variation in headsize to particular age-brackets, it was an important catalyst for future cross-sectional research.

This observation has since been refined, with brain over-growth in autism considered to be limited to the first three years of life. In a cross-sectional study of brain circumference, Hazlett et al. (2005) contrasted ASD participants with two control groups; developmentally delayed and TD children. Using
retrospective records, no differences in brain circumference were identified at birth between the groups. For children aged between 18 and 35 months, volumetric MRI revealed those with autism had a significantly larger head circumference than TD children (4.7% larger) and developmentally delayed children (6.7% larger). Increased brain volume shortly after birth in participants with ASD has been supported by a number of studies (Courchesne et al., 2001; Courchesne, Carper & Akshoomoff., 2003; Dawson, Munson, Webb, Nalty, Abbott & Toth., 2007, Schumann et al., 2010). A recent MRI study of infants aged between 6-9 months old who went on to later develop autism suggests increased brain volume may in part be characterized by extra-axial fluid, particularly in the frontal lobes (Shen et al., 2013). It has been suggested that enlarged brain size in infancy amongst individuals with autism may be exacerbated by maternal IgG autoantibodies which are reactive to the fetal brain (Nordahl et al., 2013).

Further autism research has identified that following this period of brain overgrowth, the autistic brain undergoes a period of arrested growth through childhood and adolescence. Dawson et al. (2007) analysed head circumference in children later diagnosed with autism from birth to age three. As found previously, autistic children at 12 months had a head size approximately one standard deviation greater than the norm. From 12 to 36 months however, the head growth of those with autism dipped below the rate expected of children their age by approximately one standard deviation. This finding has been supported both cross-sectionally (Courchesne et al., 2003) and longitudinally (Schumann et al., 2010).
Carper et al. (2002) provides evidence that this slowed growth continues into adolescence, as autistic children aged 4 to 12 years showed no difference in brain size compared to TD participants. Moreover a meta-analysis of 12 studies (total \(N=581\)) by Redcay and Courchesne (2005) concluded that the autistic brain is 10% larger than average at one year of age, but is only 2% larger through adolescence and 1% larger by adulthood (Figure 2.6). This growth trajectory was further confirmed in a second meta-analysis of both cross-sectional and longitudinal data comprising 586 MRI scans, with an age range of participants between 2-50 years old. (Courchesne, Campbell & Solso, 2010). Reductions in brain size are particularly pronounced in the amygdala (Pierce, Haist, Sedaghat & Courchesne, 2004) and frontal cortex (Kosaka et al., 2010).

Figure 2.6: Best fit curve from meta-analysis of 15 autism studies of head circumference and magnetic resonance imaging (MRI). The autistic brain undergoes a period of rapid development in the first year of life resulting in an abnormally large brain. Development then slows, and brain size begins return to normal size by early adulthood (Redcay & Courchesne, 2005).
2.3.7 Conclusion

In summary, although a number of brain regions have been linked to ASD, the neuroanaomtical basis of autism does not appear to be localised to any specific brain area. Although some regions such as the cerebellum and frontal lobe tend to be more reliably associated with the ASD phenotype, there is inconsistent evidence for the role of others such as the basal ganglia and hippocampus, with many null findings and lack of replication of specific findings. Nevertheless, there is accumulating evidence that ASD is linked to atypical neurodevelopment from an early age, with brain volume deviating markedly from norms of TD children. As a result, more recent approaches to the neuropathology of autism have considered the role of functional and structural networks, as opposed to distinct, anatomical regions.

2.4 The Abnormal Connectivity Theory of Autism

Traditionally, neuroimaging research has attempted to link brain structure to function. However, there is an emerging view that the neural basis of higher-order cognitive functions, such as the ability to infer others’ intentions or to comprehend metaphors, arise from synchronized activity of many cortical regions across the brain (Frith, 2004). Thus, several theorists (i.e. Belmonte et al., 2004; Just, Cherkassky, Keller and Minshew, 2004) have suggested that autism may be associated with anomalies in connectivity across the brain that interfer with integrative, neural processing (Keary et al., 2009). In this section, the various methods used to probe functional and structural connectivity in autism will be reviewed.
2.4.1 fMRI Evidence – Functional Connectivity

One means to assess cortical connectivity in the brain is *functional* connectivity (FC). FC investigates low frequency, interregional correlations between regions across the brain. One means of doing this is by correlating the average time course of all the activated voxels (volumetric pixel) from a predefined region of interest (ROI). FC can be established between a ROI and the whole brain, or with other pre-specified ROIs (Just et al., 2004). Regions with similar temporal response profiles (i.e. synchronous activation of voxels in anatomically separate but related regions) are believed to work together on a given task (Koshino et al., 2005).

At present, there is mounting evidence for abnormalities in FC among individuals with an ASD (Vissers et al., 2012). One theory suggests that participants with autism possess overall reduced connectivity (Just, Keller, Malava, Kana & Varma, 2012). The majority of evidence for this theory has examined FC whilst participants engage in specific cognitive and affective tasks, such as language (Just et al., 2004; Kana, Keller, Cherkassky, Minshew & Just, 2006), executive functioning (Just, Cherkassky, Keller, Kana & Minshew, 2007), memory (Koshino et al., 2005), visuomotor skills (Villa-Lobos, Mizuno, Dahl, Kemmotsu & Muller, 2005), and social based tasks (Koshino et al., 2008). All of these studies have demonstrated reduced FC from frontal ROIs, to more posterior brain regions in parietal, temporal and occipital cortices (for an example, see Figure 2.7).

Although some studies further linked reduced FC to poorer task performance (Just et al., 2007), other studies found that despite differences in FC, task performance was the same between TD and autism participants (Kana et al.,
The significance of reduced FC in individuals with an ASD during cognitively demanding tasks is unclear, but has been attributed to a reduction in co-ordination of brain regions when individuals with autism attend to a task (Just et al., 2012).

Another form of FC analysis known as task regression utilizes task-based experimental designs, but partials out the effect of task-dependent brain activity. This is typically completed with structural equation modelling (Fair et al., 2007), and the use of low-pass filters under 0.1 Hz. Focusing upon low-frequency signal fluctuations is believed to be a more sensitive measure of intrinsic connectivity, as these filters remove the effects of task-driven, high frequency fluctuations (Muller et al., 2011). Findings from these studies have generated mixed effects, with some reporting reduced FC (Jones et al., 2010), whilst others have reported increased FC in individuals with an ASD from the thalamus (Mizuno, Villalobos, 2006; Koshino et al., 2005).
Davies, Dahl & Muller 2006) motor cortex (Turner, Frost, Linsenbardt, McIlroy & Muller, 2006) and extrastriatal regions (Noonan, Haist & Muller, 2009).

A third method for studying FC is to use measures obtained during a ‘resting state’, where participants lie in the scanner and let their mind wander. Because these studies lack controlled experimental conditions, it is not possible to use task-regression to estimate and remove cognitively based signal changes (Muller et al., 2011). Nevertheless, band-pass filtering can still be used to minimize the impact of such fluctuations in the blood oxygen level dependent (BOLD) signal. These studies have typically investigated the default mode network, which is a baseline or ‘idling’ state of brain activity that is observed when a subject is not attending to the outside world (De Luca, Beckmann, De Stefano, Matthews & Smith, 2006; Fox & Greicius., 2010).

The default mode network is comprised of several regions including the medial frontal cortex, posterior cingulate, precuneus and medial temporal areas. Investigating FC in these regions, a number of resting-state studies report reduced connectivity in participants with an ASD by comparison to TD individuals (Assaf et al., 2010; Kennedy & Courchesne, 2008; Weng et al., 2010). However, other studies have reported a mix of under and over-connectivity (Monk et al., 2009; Paakki et al., 2010).

By looking at the present body of research in aggregate, findings are mixed, with no clear consensus in the literature regarding methodology. Studies have varied in regard to (a) low pass versus high pass filtering, (b) task activation versus task regression, and (c) whole-brain versus network specific connectivity. In a meta-analysis of 32 FC studies, Muller et al. (2011) report that all past studies that have not used low or band-pass filters have identified reduced
connectivity in those with an ASD. On the other hand, all studies combining low-pass filtering, task regression and whole-brain analyses report mixed effects, including greater FC in participants with an ASD. Thus, differences in methodological assumptions appear to have a significant impact upon the differences in FC observed between ASD and TD individuals. Future findings need to be interpreted within the context of the analytical design undertaken.

2.4.2 MRI Evidence – White Matter Volume

Another method used to investigate connectivity in autism is morphometric analysis of white matter (WM). This method allows for grosse volumes of WM to be compared between TD and ASD participants, which are involved in short and long-range structural connectivity (Kandel et al., 2004). Amaral et al. (2008) summarized the results of six studies that directly compared levels of white and gray matter between autistic cases and controls, aged between 3 and 20. Although these studies identified both white and gray matter levels as being elevated in autism compared to TD participants, in 4 of the 6 studies they found white matter levels were proportionally greater than gray matter levels. An example of one of these results reported that participants with an ASD had significantly greater cerebral white matter when adjusting for total brain volume (441.04cm³ compared to 383.94cm³) (Herbert et al., 2003).

Developmental research has examined WM abnormalities in participants with ASD from different age brackets. Carper et al. (2002) conducted a cross-sectional study that investigated differences in white and gray matter levels in ASD and TD participants from 2 to 12 years of age. ASD children aged from 2 to 3 years were found to have elevated white and gray matter compared to TD in
this age group. From 4 to 12 years of age, WM volume in the frontal lobe was found to increase by 45% in TD participants, and only 13% in ASD participants. A similar finding of arrested growth in ASD participants was made for gray matter in the frontal lobe (a 20% increase in TD participants compared to 1% in those with an ASD) and temporal lobe (17% as opposed to 1%). This is consistent with the developmental evidence that autism is associated with a period of slowed brain growth during childhood (Dawson et al., 2007; Redcay & Courchesne., 2005). Thus, there is preliminary evidence that WM volumes are abnormally high in young children with autism, where the rate of growth of these structures begins to slow relative to TD participants in late childhood and adolescence.

Research investigating inter-hemispheric connectivity has focused upon abnormalities in the corpus callosum in those with an ASD. Most research thus far has identified a smaller corpus callosum in participants with an ASD, even when comparing to developmentally delayed individuals (Boger-Megiddo et al., 2006), and adjusting for cerebrum size (Egaas, Courchesne & Saitoh, 1995; Hardan, Minshew & Keshavan, 2000; Keary et al., 2009).

A number of studies have attempted to identify whether specific sub-regions of the corpus callosum are vulnerable in autism. Piven, Bailey, Ranson and Arndt (1997) found that the middle and posterior (but not the anterior) regions of the corpus callosum were significantly smaller in ASD participants compared to those who were TD. The middle section had the greatest size discrepancy (2.85cm² to 3.00cm² diameter). To the contrary of this finding, Vidal et al. (2006) found that the only part of the corpus callosum that was significantly smaller among an autism sample was the anterior region, whilst Just et al. (2007)
found both the anterior and posterior regions to be significantly smaller in an autistic sample. These mixed results may be attributable to differences between the studies in how the corpus callosum was partitioned. Collectively however, these studies provide preliminary evidence of a smaller corpus callosum in ASD.

2.4.3 DTI Evidence – Structural Connectivity

Although FC allows inferences to be made about the functional relationships between brain regions, this technique is unable to probe into underlying structural differences in connectivity (Sundaram et al., 2008). Similarly, although volumetric studies can reveal differences in the size of brain structures, they don’t reveal anything about the organization of WM tissue (Tavers et al., 2012). A method that goes some way to overcome these limitations is Diffusion Tensor Imaging (DTI); an MRI technique can identify differences in microstructural and macroscopic organization of WM (Langen et al., 2010).

The membranes of axons and myelin constituting WM, cause the diffusion of water perpendicular to WM tracts (radial diffusivity) to decrease relative to directions parallel to WM (axial), leading to anisotropic water flow (Lee et al., 2007). This flow is represented by three eigenvalues ($\lambda_1, \lambda_2, \lambda_3$), which reflect the length of each eigenvector (Travers et al., 2012).

In DTI, several measures can be extracted. Fractional Anisotropy (FA) is a normalized value ranging from 0 to 1, which represents the fraction of the tensor that can be assigned to anisotropic diffusion (Jones, 2008). Mean Diffusivity alternatively is the average radius of the diffusion tensor ellipsoid and is sensitive to the density of tissue barriers in all directions (Travers et al., 2011).
Examining the eigenvalues separately is argued to provide a more complete picture of WM structure (Song et al., 2005). Axial Diffusivity (AD) looks at water diffusivity parallel to WM tracts ($\lambda_1$), whilst Radial Diffusivity (RD) expresses water diffusivity perpendicular to tracts ($[\lambda_2 + \lambda_3]/2$). RD is believed to be sensitive to dysmyelination and demyelination (Harsan et al., 2006), whilst AD is sensitive to axonal injury (Travers et al., 2012).

Concerning ASD, the most common finding to arise from analyses of diffusion data has been reductions in FA (Barnea Goraly et al., 2010; Jou et al., 2011; Kumar et al., 2010; Lee et al., 2007; Noriuchi et al., 2010; Pardini et al., 2009; Shukla, Keehn, Lincoln & Muller, 2010; Thakkar et al., 2008), and increases in MD (Cheon et al., 2011; Kumar et al., 2010; Shukla, Keehn, Lincoln & Muller, 2010), distributed widely across the brain. WM tracts commonly associated with reduced FA in participants with ASD include the superior longitudinal fasciculus (SLF), cingulum bundle, uncinate fasciculus (UF) and the corpus callosum (Barnea-Goraly et al., 2010; Jou et al., 2011; Kumar et al., 2009; Pardini et al., 2009; Shukla et al., 2010; Thakkar et al., 2010).

There are also reports of increased FA in ASD samples (Billeci et al., 2012; Cheng et al., 2010; Cheung et al., 2009; Weinstein et al., 2011), which is typically attributable to younger cohorts of participants. However, other studies utilizing adult samples have demonstrated patterns of increased and decreased FA in autism (Sayhoun, Belliveau & Mody, 2010), and no difference to TD participants (Brito et al., 2009). Additionally, there are reports of increased RD (Amies et al., 2010; Shukla et al., 2010), and decreased AD (Barnea-Goraly et al., 2010). Clearly, the distribution of WM anomalies in participants with ASD
remains heterogeneous, and there is a need to identify more specific WM disturbances linked to the disorder.

A past theory (Hier, LeMay & Rosenberger, 1979) that has received renewed interest since the advent of DTI is that participants with ASD demonstrate greater left hemisphere impairment. Among typically developing (TD) participants, there is a trend toward increased FA in the arcuate and uncinate fasciculi of the left hemisphere (Catani et al., 2007). In participants with an ASD, these two structures have been found to possess reduced lateralization, demonstrating greater impairment than the right hemisphere using ROI based analyses (Fletcher et al., 2010; Langen et al., 2011; Nagae et al., 2012) and diffusion spectrum imaging (Lo et al., 2011). Moreover, a recent meta-analysis of six previous DTI studies reported that participants with ASD demonstrate significantly reduced FA in the left, but not right SLF and UF (Aoki, Abe, Nippashi & Yamasue, 2013).

2.4.4 Conclusion and Future Research

The precise reason why participants with autism demonstrate atypical connectivity remains unknown. Recent evidence suggests it could be due to spontaneous changes in the genome through no mode of inheritance (Sebat et al., 2007), and disturbed neural migration during gestation (Blaylock, 2008; Schmitz & Rezaie, 2008). Adding weight to the abnormal neural migration theory is the finding that ASD participants demonstrate more abnormalities in the gene RELN, which plays an important role in cortical layering (Fatemi et al., 2001).

Problems with neural migration in participants with an ASD may have a flow on effect upon neurodevelopment at critical developmental periods. It has
been suggested that atypical neural migration may cause retention of subplate neurons, which further leads to abnormalities at white-gray matter boundaries (Avino & Hustler, 2010). Furthermore, as previously reviewed, there is evidence from post-mortem studies that participants with ASD possess disorganized minicolumns, including a narrower width and more densely packed neurons (Buxhoeveden et al., 2006; Buxhoeveden et al., 2002; Casanova et al., 2006).

Nevertheless, there are questions requiring further research for theories of abnormal connectivity in participants with an ASD. For instance, there is a need for more research into connectivity of specific, *a priori* brain networks. Among participants with an ASD, FC and DTI research has provided evidence of both increased and decreased connectivity, spanning across the whole brain. Thus, it remains possible that certain patterns of functional and structural connectivity in ASD may be tied to particular networks. Thus, the final section of this literature review will consider recent research that has implicated one such neural network into the pathology of ASD.

### 2.5 The Mirror Neuron Theory of Autism

“Every mental representation of a movement awakens to some degree the actual movement which is object” (William James, 1890).

#### 2.5.1 Definition of Mirror Neurons

A recent approach to understanding the pathogenesis of autism has been dysfunction of the mirror neuron system. Mirror Neurons (MNs) are a class of
visuomotor cells that not only discharge when an individual performs a particular action (such as reaching for a piece of food), but also when an individual watches somebody else perform a similar action (such as a friend reaching for a piece of food) (Rizzolatti & Craighero, 2004).

In monkeys, MNs require an interaction between biological effectors such as fingers or mouth, and an external object such as food (Rizzolatti & Craighero, 2004). In humans, there is evidence that MNs may respond to \textit{intransitive} or mimed actions (Gallese, Keysers & Rizzolatti, 2004), and to the sound of an action when the participant does not see the action (Kohler et al., 2002; Oberman, Pineda & Ramachandran, 2007), observing an individual being touched (Keysers et al., 2004), and listening to linguistic material (i.e. \textit{she grasped the apple}; Fogassi & Ferrari, 2007).

\subsection*{2.5.2 Anatomy of Mirror Neurons}

In monkeys, MNs are believed to constitute a minority of cells in frontal and parietal lobes. The first single cell study conducted on macaque monkeys by Gallese, Fadiga, Fogassi and Rizzolatti (1996) identified that 17\% of neurons in the premotor cortex (92 of 532) possessed mirror properties (Figure 2.8). Subsequent studies have found similar distributions of MNs in area F5 of the inferior frontal gyrus (IFG), and area PF of the inferior parietal lobule (IPL) (Rizzolatti, Fadiga, Gallese & Fogassi, 1996; Rizzolatti & Luppino, 2001).

In humans, the distribution of MNs in the brain is less clear. Research using neuroimaging techniques such as fMRI (Binofski & Buccino, 2006; Buccino et al., 2002; Buccino et al., 2004; Grezes, Armony, Rowe & Passingham, 2003) and neurophysiological research using Electroencephalograph
(EEG) (Perry & Bentin, 2009) and Transcranial Magnetic Stimulation (TMS) (Fadiga, Fogassi, Pavesi and Rizzolatti., 1995; Heiser, Iacoboni, Maeda, Marcus & Mazziotta et al., 2003) provide indirect evidence for the existence of MNs in areas homologous to macaque monkeys. These are the premotor cortex, IFG (corresponding to the *pars opercularis*) and the IPL (corresponding to the supramarginal gyrus). The Superior Temporal Sulcus (STS) is also part of this network, and is believed to encode for biological motion (Aziz-Zadeh et al., 2006; Iacoboni et al., 2001; Kaplan & Iacoboni, 2007).

Nevertheless, there is minimal direct evidence for the existence of MNs in humans. Mukamel, Ekstrom, Kaplan, Iacoboni and Fried (2010) conducted the only one single cell study on humans by investigating patients with intractable epilepsy, and report neurons with mirror properties in the medial frontal cortex supplementary motor area and the medial temporal lobes (hippocampus parahippocampal gyrus and entorhinal cortex). Although neurons with mirror properties were also observed in the amygdala, pre-supplementary motor area and both rostral and dorsal aspects of the ACC, the number of such cells in these

![Figure 2.8. Response of a single mirror neuron in area F5 of the Macaque Monkey. The bars on the X axis represent an action potential by the neuron in response to stimulus. The activity to the left is the monkey’s response to the experimenter’s hand grasping a piece of food. The activity to the right is the monkey’s response to itself grasping the food (Gallese, Fadiga, Fogassi & Rizzolatti, 1996).](image_url)
regions did not reach significance. The percentage of neurons that responded to both execution and observation of hand movements ranged from 10% in the supplementary motor area, to 23% in the parahippocampal gyrus.

A key implication of this single cell study is that MNs may be distributed in more regions across the brain in humans. Thus, the prevailing view of a fronto-parietal circuit homologous to macaque monkeys may be limited (Keysers & Gazzola, 2010). Several depth electrode studies in Rhesus Monkeys demonstrate evidence of MNs in the lateral intraparietal area (Shepherd, Klein, Deaner & Platt, 2009), primary motor cortex and dorsal premotor cortex (Cisek & Kolaska, 2009; Dushanova & Donoghue, 2009; Tkach, Reimer & Hatsopolous, 2008).

In humans, two meta-analyses of fMRI studies have been conducted to further quantify regions attributed to MNs. Firstly, Caspers, Zilles, Laird and Eickhoff (2011) conducted a meta-analysis of 104 action observation experiments, comprising 1061 TD participants. This review revealed a number of macro-anatomic locations including dorsal and medial premotor cortex, fusiform gyrus, lateral occipital gyrus and supplementary motor area, in addition to the aforementioned fronto-parietal network. Secondly, a meta-analysis of 125 fMRI studies by Molenberghs et al. (2012) reported that mirror activity in humans had been attributed to 34 different Brodmann areas. The two most common anatomical regions attributed to MNs were BA40 (N=60), and BA6 (N=59). However, this analysis had less-discerning selection criteria than did Caspers et al. (2011). More generally, it must be acknowledged that fMRI is limited in its ability to probe MNs (Glenberg, 2011). As it stands, the distribution of MNs in the human brain requires more evidence, but appears to form a network of prefrontal, motor, inferior parietal and temporal areas.
2.5.3 Functions of Mirror Neurons

The essential function of MNs in lower-order primates and humans appears to be action understanding. However, this assertion has not gone unchallenged (i.e. Dinstein, 2008). Fabbri-Destro and Rizzolatti (2008) argue that MNs discharge to the goal of motor actions, regardless of the effector used and movements made to accomplish it. Thus, MNs go beyond encoding for kinematic aspects of sensori-motor behavior, and contribute to the recognition of other people’s actions (Oberman et al., 2005). From this basis, further research has been conducted to explore whether MNs have a role in more complex abilities such as imitation, discriminating emotions, and understanding intentions. Pertinent to this review, these abilities are commonly disturbed in those with an ASD.

There is a small body of evidence that MNs may contribute to imitation, and imitative learning (Wiedermann, 2012). One neuroimaging study reports that the *pars opercularis* was active during observation, execution, imagination and imitation of basic motor acts (Iacoboni et al., 1999). A more discerning experiment by Heiser et al. (2003) used repetitive transcranial magnetic stimulation to disrupt cortical activation in the *pars opercularis*. They found that TD participants made more errors imitating compared to a control task whilst this region was compromised. In this study, it is unclear if task complexity contributed to an increased error rate, as opposed to the specific demands of imitation. Although several other studies support the link between MN areas and imitation (Iacoboni et al., 2001; Nishitani & Hari, 2000), there is not enough evidence yet to determine if MNs specifically are involved in this ability.
MNs have been suggested to contribute to empathy (Wolf, Gales, Shane & Shane, 2001). It has been theorized that when viewing someone in an emotionally distressing situation, MNs are the basis for an observer to experience an ‘inner-simulation’ of that individual’s emotional state (Corradini & Antonietti, 2013; Rizzolatti & Sinigaglia, 2006). Moreover, the MN network and STS possess anatomical links to limbic structures involved in emotion, by way of the dysgranular field of the insula (Augustine, 1996). Consistent with these anatomical findings, Carr, Iacoboni, Dubeau, Mazziotta and Lenzi (2003) used fMRI to determine what brain regions would demonstrate increased BOLD in response to observing emotional faces, and identified a network consisting of the pars opercularis, STS, insula and amygdala.

Furthermore, several studies have identified insula neurons with mirror-like properties. Wicker et al. (2003) conducted fMRI scans while participants inhaled a foul smelling odorant, and watching a video of someone emotionally expressing disgust, and identified insula activity in both conditions. Several other studies have made similar findings (Krolak-Salmon, 2003; Phillips et al., 1997). Similarly, neurons in the anterior cingulate cortex have been found to discharge in response to pain, and observing someone else in pain (Ramachandran & Oberman, 2007). This data provides preliminary evidence that the insula and anterior cingulate demonstrate mirror like activation in response to empathic stimuli.
Figure 2.9: Unit 67 and 87 are examples of Area PF mirror neurons, selective for the goal of the motor action. The red bars represent the moment the monkey begins moving its arm to grasp the object. In the action execution condition, neuron 67 would begin to discharge during the arm movement when grasping to eat, but would fail to discharge during the same arm movement when grasping to place. In the action observation condition, neuron 87 would discharge more strongly when observing the experimenter grasping an object to eat, by comparison to grasping to place (Figure adapted from Fogassi et al., 2005).

In monkeys, MNs have been found to respond to the specific intention of a performed or observed action. Fogassi et al. (2005) identified that the majority of grasping MNs (N=165) studied in the IPL of Macaques discharged only if this action was followed by a specific intent. For instance, one such neuron discharged when a grasping action was followed by bringing a piece of food to the mouth, but was near absent if this action was followed by placing (Figure 2.9). This discharge pattern applied to both action execution and observation, and was not influenced by what the object was.

This finding prompted research into the role of MNs in human intention understanding (Jellema, Baker, Wicker & Perrett, 2000). At present, there is a paucity of research on intentions in humans due to the inherent difficulty of defining motor-intentions (Bonini, Ferrari & Fogassi, 2013; Jellema, Baker, Wicker & Perrett, 2000). Nevertheless, using fMRI, Iacoboni et al. (2005) studied whether the same grasping action would elicit a different pattern of activity based
upon an individual’s intention. Intention was measured by having participants view a grasping action where the intention of the action was embedded within the context (an intention to drink versus an intention to clean, see Figure 2.10). An increase in BOLD signal in the IFG was found in response to the drinking intention by comparison to the cleaning intention, indicating a distinct signal response for different intentions in MN regions. Subsequent research has identified that the right hemisphere is particularly important for encoding intentions, regardless of whether mirror or higher-order visual mechanisms are recruited (Ortigue, Sinigaglia, Rizzolatti & Grafton, 2010).

Figure 2.10: The two conditions under which MN activity in response to intention was measured. The context condition established whether any difference in activity was observed for a scene depicting ‘before tea’ and ‘after tea’. The intention condition assessed two types of contexts surrounding a grasping action. The ‘before tea’ context indicates the intention to drink, whilst the ‘after tea’ context indicates the intention to clean up (Iacoboni et al., 2005).

A link has been made between MNs and speech and communication abilities (Rizzolatti & Arbib, 1998). In humans, the pars opercularis is believed to be an important node in the mirror network. This region forms part of Broca’s
area; a region involved with phonology and semantics in addition to motor functioning. Thus, perhaps not surprisingly, this region, along with the IPL has demonstrated increased BOLD response when observing gestures with communicative significance (Montgomery, Isenberg & Haxby, 2007).

A limited body of research suggests that when simply listening to verbal stimuli, speech related motor areas become active. One such study by Fadiga, Craighero, Buccino and Rizzolatti (2002) used TMS to stimulate the left motor cortex whilst participants listened to words with a double ‘f’ (which requires slight tongue mobilization) or double ‘r’ (which requires a movement of the tongue). Motor evoked potentials from tongue muscles revealed that the double ‘r’ condition had more activity, consistent with its need for stronger activation when pronounced. Collectively, MN research on imitation, empathy, intentions and speech has led to theories that MNs, along with the vocal apparatus of humans may have had an important role in the evolution of language (Fogassi & Ferrari, 2012; Perlovsky & Ilin, 2013).

2.5.4 Evidence for Mirror Neuron dysfunction in Autism

Prior to the discovery of MNs, Rogers and Pennington (1991) theorized that autism may be characterized by a deficit in self-other matching. This ability involves forming and coordinating social representations of the self and others. Understanding others behaviours and social rules is achieved by extracting patterns of similarity between the self and other. Williams, Whiten, Suddendorf and Perrett (2001) recognized that this theory of autism was similar to the key role of MNs, and suggested that disruption of MN functioning may contribute to this self-other matching deficit.
In 1999 a research group from the University of California and another from the University of St. Andrews independently suggested that disturbance of MNs may be linked to autism (Oberman et al., 2005; Williams, Whitten, Suddendorf & Perett, 2001). Although a controversial theory that has been subject to criticism, (Hamilton, Brindley & Frith, 2007), this was based upon MNs functional significance in imitation, empathy and language development. Thus, it has been suggested disturbance of MNs may contribute to these symptoms in autism (Ramachandran & Oberman, 2007).

2.5.4.1 EEG Research

A method to investigate MN activity in humans is electroencephalogram (EEG). EEG can measure a band of electrical activity in the brain known as the mu wave, which reflects large amplitude oscillations of the synchronized activity of sensorimotor neurons (Oberman et al., 2005). It has been well established that the mu wave is suppressed by input from premotor and inferior parietal neurons, when performing a volitional movement (Muthukumaraswamy & Johnson, 2004; Arnstein, Cui, Keysers, Mauritz & Gazzola, 2011). Interestingly, mu suppression is also found when participants passively observe another individual performing a motor action (Ramachandran & Oberman, 2007). Thus, when an individual observes a motor action, it is possible that the block in mu wave activity is attributable to input from premotor MNs.

If MNs are in some way abnormal in those with autism, mu wave suppression may be absent or reduced when observing motor actions due to faulty inputs from the premotor cortex. Using this methodology, Oberman et al. (2005) compared HFA and TD participants whilst they watched a bouncing ball
(control condition), a hand moving (observation condition), or moving their own hand (execution condition). They found TD participants showed significant mu wave suppression during observed and performed hand movements, whilst HFA participants only showed significant suppression during performed hand movements (Figure 2.11). Subsequent studies have replicated this finding using hand actions (Martineau, Cochin, Magne & Barthelemy, 2008), observing faces (Bernier, Dawson, Webb and Murias, 2007) and testing the familiarity of the person being observed (Oberman, Ramachandran & Pineda, 2008).

Figure 2.11: Mu wave suppression in control and ASD participants when watching balls, watching hands (MN condition) and moving own hands conditions. Bars represent mean log power in mu frequency, where a value less than zero indicates suppression. C3, CZ and C4 are scalp locations. Significant suppression was observed in both groups for moving own hands condition. In the watching hands condition, mu wave suppression was not significant in the ASD group, suggesting MN activity was greatly reduced. *p<.05, **p<.01, ***p<.005 (Oberman et al., 2005).

Nevertheless, EEG studies of mu wave in autism have also revealed null findings. Using a larger sample (20 participants in each group compared to approximately 10 in past studies), Fan et al. (2010) found no difference in mu suppression between TD and ASD participants when they observed basic hand actions. Likewise, with a sample of age and developmentally matched participants, Raymaekers, Wiersema, Roelf and Roeyers (2009) found no
difference in \( \mu \) suppression between participants with HFA and TD when observing and executing basic hand actions.

2.5.4.2 fMRI and MRI Research

fMRI has allowed for research to investigate activation in mirror regions with greater spatial acuity. To date, three studies have examined emotion or face-based stimuli, and reported a reduced BOLD response in MN areas among those with autism. Dapretto et al., (2006) found that TD participants demonstrated significant activity in the \textit{pars opercularis} during imitation and observation of expressive faces. For individuals with HFA, activity in the \textit{pars opercularis} was significantly reduced during observation. Using a similar paradigm that required participants to observe expressive faces, Hadjikhani et al. (2004) found reduced activation in the inferior frontal cortex and superior temporal gyrus of individuals with HFA compared to TD participants. Bookheimer, Wang, Scott, Sigman, & Dapretto (2006) also reported reduced activity in autistic individuals when observing faces, but only specified this reduction was in the prefrontal cortex.

fMRI paradigms requiring participants to observe hand gestures have produced mixed results. Using an imitation paradigm, Williams et al. (2006) found that individuals with an ASD demonstrated greater activation in the ventral premotor cortex than did TD individuals. Of interest, they did not identify activity in the IFG for either group. However, this could be a potential false negative result. In this study, the field strength of the scanner was 1.5 T. Research to have directly compared 1.5 and 3 T scanners has demonstrated that 1.5 T systems demonstrate significant signal losses in regions which include the
inferior frontal gyrus (Krasnow et al., 2003). Thus, field strength may have been responsible for absence of inferior frontal gyrus activity in this study.

Similarly, Martineau, Andersson, Barthelemy, Cottier and Destrieux (2010) found participants with an ASD had stronger activation in the *pars opercularis* bilaterally than TD individuals when observing simple hand gestures. This provides limited evidence that individuals with an ASD demonstrate either equivalent or hyper-activation in frontal regions believed to possess MNs when observing hand-gestures.

A number of other hand based paradigms report null findings in mirror regions between ASD and TD participants. Dinstein, Thomas, Humphries et al. (2010) compared participants with autism to TD individuals whilst they observed and executed basic hand actions, and found no difference between the groups in MN areas including the intra-parietal sulcus and inferior frontal gyrus. Likewise, Marsh and Hamilton (2011) contrasted observation of hand movements with non-biological motion, and found no difference between ASD and TD participants.

An MRI study by Hadjikhani, Joseph, Snyder and Tager-Flusberg (2006) examined brain size of MN areas in TD and ASD subject. They found the ASD group had thinner gray matter in the *pars opercularis*, IPL and STS with known functioning in social cognition (such as the anterior cingulate), no other regions were significant. The authors argue that the cortical thinning they observed is part of a broader deficit in networks involved in social cognition.

Based on this small literature, there appears to be a trend that those with autism demonstrate reduced activity in MN areas when observing emotionally expressive faces. In regard to hand-based paradigms, the evidence is less clear, with research reporting either no difference to TD participants, or increased
frontal activation in individuals with an ASD. As the present research base is only small, more research is required to determine whether differences reported in the activation of MN regions is task based in participants with an ASD.

2.5.4.3 Criticism of the Theory

There are theoretical issues that need to be considered when evaluating the MN hypothesis of autism. Southgate and Hamilton (2008) point out that often differences in BOLD response are observed between TD and ASD participants, but this does not translate into cognitive differences. For instance, Dapretto et al. (2006) report different activation in the pars opercularis between these groups, but no actual differences in imitation ability. Thus, it may be that these complex abilities do not have a critical dependence upon MN regions. Nevertheless, it must be acknowledged that anomalies in imitation amongst participants with autism have been previously established, and may not be captured in a laboratory situation. Further, more subtle imitative anomalies such as echopraxia or echolalia may have been present, but not captured in the present paradigm.

There are also methodological considerations when addressing the variable findings in the literature. Firstly, most fMRI, EEG and TMS studies investigating MNs and autism possessed small sample sizes, with an N under 10 in each group (i.e. Martineau et al. 2010). Secondly, the precise anatomical location of MNs in humans is poorly defined, meaning a priori hypotheses have been difficult to develop. For example, some studies (i.e. Grezes et al., 2003; Williams et al. 2006) fail to find frontal activation in TD participants during MN tasks. As mentioned however, this may be attributable to insufficient signal strength in 1.5 T scanner systems.
A third and important point is that during observation paradigms, studies have varied considerably in what control conditions are used. Examples include a non-moving hand (Martineau et al., 2010), geometric patterns (Marsh & Hamilton, 2011) and a blank screen (Dinstein et al., 2010). The type of control condition used is likely to influence the results, meaning all past findings need to be interpreted within this context. Comparisons between studies with different designs need to be made cautiously.

2.6 Conclusion

In sum, despite research identifying numerous biological correlates with autism, the actual causes of the disorder remain elusive. Although evidence suggests autism is inherited, the specific genetic anomalies are not clear. The closest linkage is a GABA system gene known as GABRB3 at chromosome 15q11-13. However this gene only accounts for approximately 1 to 3% of variance in the disorder. It is highly likely autism is an oligogenic disorder, where 100s of genes confer risk to its development. Teratogens may contribute to a minority of autistic cases, but are not a key cause, with most evidence linking autism to vaccines being discredited. Recent advances in epigenetics may help explain the higher male prevalence, and address how environmental factors interact with genes to cause the autism phenotype.

Autism is related to a diverse range of neurophysiological abnormalities in activation, volume and cell density, particularly in the frontal lobes and cerebellum. There is growing evidence that these abnormalities in autism may stem from an abnormal developmental trajectory. Data suggests that atypical
development in autism begins with a smaller brain at birth, then a larger brain in early childhood, where brain growth begins to arrest through adulthood and adolescence. Atypical brain development in autism is likely to results in brain-wide differences in neural communication.

Thus, more recent theories of the neurobiological basis of autism postulate problems in connectivity and organization in the brain. Studies of FC have identified patterns of increased and decreased connectivity in the brain, where findings depend upon the methodology employed and brain regions studied. Research using DTI provides evidence of impaired structural connectivity, particularly in the left hemisphere. Overall, deficits in connectivity have been predominantly situated in the frontal lobe, but have not often been guided by a priori specified networks.

Thus, a recent network hypothesized to function anomalously in autism is the MN network. MNs are believed to be important for imitation, empathy, TOM and language development; all key areas of autism deficit. Although only a small amount of research has been conducted thus far, evidence utilizing EEG, fMRI, and TMS suggests that MN functioning is abnormal in some autistic cases, with emotion based tasks revealing reduced activation, and hand-action based tasks revealing increased activation in autism samples. As of writing this, there are no studies to have thoroughly investigated both functional and structural integrity of the MN system in a single autism sample.
2.7 Research Question for this Thesis

The subjective, symptom-based nature of ASD stands as an impediment toward the development of coherent and effective diagnostic criteria. For this reason, identification of neural markers that are associated with ASD or even a small population with the disorder is a research priority. Consequently, this thesis intends to use a multi-faceted approach to examine the MN hypothesis of autism, to assess the degree to which this network can be linked to the disorder. To do this, three neuroimaging techniques will be implemented, and examined within two groups (TD and autism).

Using fMRI, Study 1 intends to examine if stimulus-induced differences in activation of MNs regions exist between TD and autism participants. A block design will be implemented to evaluate the BOLD response of MN regions whilst participants observe basic hand gestures. Study 2 will also utilize fMRI, but contrast FC of MN regions between the two groups. This will be completed by seeding MN regions and examining whole-brain FC, whilst participants undertake a resting-state scan. It is hoped this will help clarify if MN anomalies in autism can be further characterized by anomalous inter-regional correlations within the fronto-parietal network. Using DTI, Study 3 will examine whether there are structural abnormalities in WM pathways that link fronto-parietal MN regions. A final chapter will provide an overview of the three studies, and attempt to integrate the results into a coherent account of how the MN network is linked to ASD. It will also address explanations for the findings that go beyond MN theory.
Although many studies have examined the function and structure of MNs in participants with autism, to this author’s knowledge, this has not been systematically investigated in a single sample using these three techniques. Thus, it is hoped this thesis will provide (a) a better understanding of anomalies in MN regions in autism, (b) provide further clarity on whether the MN system can be considered a neural marker associated with ASD, and (c) explore other functional and structural anomalies in the brain associated with autism.
Chapter 3: Response of mirror neuron regions in autism during action observation
3.1 Introduction

An important issue confronting clinicians and researchers of Autism Spectrum Disorders (ASD) is the absence of definable and reliable, neurophysiological markers that underlie the disorder. At present, the autism phenotype is broad and ill defined, with diagnosis made on the basis of behavioural symptoms. In turn, this limits the capacity for ASD to be identified early and accurately. However, in 1999, two research groups (Oberman et al., 2005; Williams, Whitten, Suddendorf & Perett, 2001) independently proposed that a class of visuomotor neurons known as Mirror Neurons (MNs) might be a candidate biomarker to ASD, and contribute to some of the key symptoms of the disorder.

MNs can be distinguished from other motor neurons by not only discharging when an individual performs a particular action, but also during observation of another being performing an equivalent action (Rizzolatti & Craighero, 2004). Examples of such actions include hand movements such as grasping, and mouth movements such as eating. Generally, MN activity is considered to be goal-selective, even if the action is only partially seen (Gallese & Sinigaglia, 2011) is intransitive (i.e. not object-directed) or even heard but not seen (Kohler et al., 2002). This characteristic of goal-selective activation occurs regardless of what movements are made to accomplish an end-goal (i.e. twisting or pulling a screw). Thus, MNs go beyond simply encoding for kinematic aspects of sensori-motor behaviour (Fabrri-Destro & Rizzolatti, 2008; Keysers & Gazzola, 2011; Keysers, Thioux & Gazzola, 2013).
Although the majority of evidence for MNs existence comes from studies on Macaque Monkeys (i.e. Gallese, Fadiga, Fogassi, & Rizzolatti, 1996), neuroimaging techniques such as functional magnetic resonance imaging (fMRI) (Binofski & Buccino, 2006; Buccino et al., 2001; Buccino, Binokski & Riggio, 2004; Grezes, Armony, Rowe & Passingham, 2003), and neurophysiological measures such as Transcranial Magnetic Stimulation (Fadiga, Fogassi, Pavesi & Rizzolatti, 1995; Heiser, et al., 2003) and Electroencephalograph (EEG) (Perry & Bentin, 2009) provide indirect evidence for a fronto-parietal network of MNs in humans. This network is believed to be homologous to Macaque mirror regions, and consists of the premotor cortex (PMC), pars opercularis of the Inferior Frontal Gyrus, Inferior Parietal Lobule (IPL) and Superior Temporal Sulcus (STS).

More recent single cell evidence suggests the distribution of MNs may go beyond the fronto-parietal network. Several depth electrode studies in Rhesus Monkeys demonstrate evidence of MNs in the lateral intraparietal area (Shepherd, Klein, Deaner & Platt, 2009), primary motor cortex and dorsal premotor cortex (Cisek & Kolaska, 2009; Dushanova & Donoghue, 2009; Tkach, Reimer & Hatsopolous, 2008). Moreover, the only single-cell study to be conducted on humans provides evidence of neurons with mirror properties in supplementary motor areas, and medial temporal areas, in addition to a non-significant number in the anterior cingulate cortex (ACC) (Mukamel, Ekstrom, Kaplan, Iacoboni & Fried, 2010), consistent with some fMRI investigations (Gazzola & Keysers, 2009).

The goal-selective nature of MNs has led to a suggestion that they may be a neural basis for simulation theories of action understanding. When a person
observes another being perform a motor action, the MN response may allow them to retrodict that person’s mental states, on the basis of their own preferences, desires and beliefs (Gallese & Singaglia, 2011). Further to this, the activity of MNs may be an efficient means to establish links between an observed action, and other functionally related actions (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Rizzolatti, Fabbri-Destro & Cattaneo, 2009). These claims are subject to ongoing debate (i.e. Dinstein, 2008; Gallese & Sinigaglia, 2011), with some doubt cast upon the plausibility of MNs to underlie such complex skills (Hicock, 2009).

Nevertheless, subsequent research has elaborated upon these initial findings. There is some evidence that MNs may contribute to socio-cognitive abilities such as imitation (Heiser, Iacoboni, Maeda, Marcus & Mazziotta et al., 2003), intention understanding (Iacoboni et al., 2005) and empathy (Wicker et al., 2003). These abilities are commonly observed as areas of deficit in individuals with an ASD – which has prompted research to investigate whether MNs are linked to autism.

Using EEG, a small body of research suggests dysfunction of premotor and parietal MNs in participants with ASD. It has been well established that an EEG band known as the *mu* wave is suppressed by input from premotor and inferior parietal neurons, both when performing or observing movement (Arnstein, Cui, Keysers, Mauritz & Gazzola, 2011; Gaustaut & Bert, 1954; Muthukumaraswamy & Johnson, 2004). This input during execution and observation which suppresses the *mu* wave is considered an indirect measure of MN activity.
Several authors studying hand actions (Martineau, Cochin, Magne & Barthelemy, 2008; Oberman, et al., 2005; Oberman, Ramachandran & Pineda, 2008) and facial stimuli (Bernier, Dawson, Webb and Murias, 2007) found that this suppression is reduced or absent in participants with an ASD, which is attributable to dysfunctional MNs. However, two more recent studies report no difference in mu rhythm attenuation between TD and autistic participants (Fan et al., 2010; Raymaekers, Wiersema & Roeyers, 2009). Although it has been argued that null findings may be the result of mirror functioning improving with age in ASD, this idea has been challenged. A recent EEG study of 117 participants (51 with autism) with an age-range of 6-17, identified that age-related increases in mu suppression occur equally in both groups (Oberman et al., 2013).

fMRI has permitted comparisons between ASD and TD participants during MN tasks with greater spatial acuity than EEG. Typically, fMRI research will utilize a block design to contrast observation of biological movement with a rest condition. Using this methodology, three studies have examined emotion or face-based stimuli, and report a reduced Blood Oxygen Level Dependent (BOLD) response in MN areas among those with autism in the pars opercularis (Dapretto et al., 2006), inferior frontal gyrus and STS (Hadjikhani et al., 2007), and prefrontal cortex (Bookheimer, Wang, Scott, Sigman & Dapretto, 2006).

Using fMRI, a further four studies have investigated hand-based gestures, and produced mixed results. Two studies provide evidence of an increased cortical response in the pars opercularis during observation (Martineau, Andersson, Barthelemy, Cottier & Destrieux, 2010), and ventral PMC during imitation (Williams et al., 2006), whilst, two further studies report no difference between the groups (Dinstein et al., 2010; Marsh & Hamilton, 2011).
Based on this small literature, there appears to be a trend that those with an ASD demonstrate reduced activity in MN areas when observing emotionally expressive faces, and either an increased or equivalent response to TD participants during hand-based paradigms. Although there are many factors that contribute to inconsistent findings such as symptom profile, scanner parameters and variation in regions of interest, one pertinent reason to this study is that hand-based paradigms have differed in what control conditions are implemented. In the hand-based paradigms outlined above, examples include a non-moving hand (Martineau et al., 2010; Williams et al., 2006), geometric patterns (Marsh & Hamilton, 2011) and a blank screen (Dinstein et al., 2010). Thus, comparisons between studies need to be interpreted within this context.

Using fMRI, the first study of this thesis will contrast the BOLD response of participants with High Functioning Autism and Asperger’s Syndrome (HFA/AS) to TD individuals during observation of hand-based actions. Similarly to Martineau et al. (2010), and Williams et al. (2006), hand actions will be contrasted with still images of a non-moving hand. This will permit valid comparisons between the results of this study, and previous work that has reported an increase in BOLD of MN regions in participants with an ASD. For all analyses, MN regions have been specified \textit{a priori}, in pre-frontal (\textit{pars opercularis}, PMC), parietal (IPL) and temporal (STS) areas. On this basis, two hypotheses were generated for this study.

\textbf{H1:} In regard to the within-groups analysis, it is hypothesized that contrasting hand-actions with a non-moving hand will reveal a significant BOLD response in MN regions for both groups.
H2: In regard to the between groups analysis, it is hypothesized that HFA/AS participants will demonstrate increased BOLD in frontal, parietal and temporal MN areas by comparison to TD participants.

3.2 Method

3.2.1 Participants

This study received ethics approval from the Deakin University Human Research Ethics Committee (DURHEC 135-2009). As the National Health and Medical Research Council covered this ethics approval, it also conformed to the Austin and Repatriation Medical Centre Ethics Committee, which covers fMRI scans at the Melbourne Brain Centre (Austin Health, Melbourne, Australia).

The present study investigated 24 adolescent and adult males, comprised of 12 TD participants, and 12 individuals who had been diagnosed with either High-Functioning Autism or Asperger’s Syndrome (HFA/AS). A clinical psychologist experienced in the assessment of ASD confirmed diagnosis using DSM-IV-TR criteria (APA, 2000). Participants with HFA/AS were age stratified to a TD participant within 3 years (Table 3.1). An independent samples t-test revealed no significant difference between the groups in participant age ($p=.47$), suggesting differences in age did not impact upon the results. In both groups, 10 of 12 (83%) were right handed. In the HFA/AS group, four of 12 possessed a comorbidity, which included anxiety, depression and tactile defensiveness, with two participants taking anti-depressants, and one taking beta-blockers. Further, three of 12 in this group had a family member with autism (in all three cases a brother, and in one case also a father).
TD participants were recruited by word of mouth. Participants with HFA/AS were recruited from various autism support organizations (i.e.: Autism Victoria) and specialist schools (Western Autism), advertisements, mail outs and from paediatric clinics. All participants gave written consent to participate in this study. For those participants under the age of 18, a parent or guardian gave written consent. One participant in the HFA group wore glasses, which were unable to be taken into the scanner. Consequently, this subject wore goggles with corrective lenses that were checked for prescription matching. For those participants under the age of 18, a parent or guardian gave written consent. Participants received a small monetary incentive to take part in this research.

Table 3.1. Participant characteristics for High-Functioning Autism and Asperger’s Syndrome (HFA/AS) group and Typically Developing (TD) participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HFA/AS (N=12)</th>
<th>TD (N=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>12 / 0</td>
<td>12 / 0</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>19.75 ±4.93</td>
<td>18.50 ± 2.50</td>
</tr>
<tr>
<td>Age (range)</td>
<td>16-30</td>
<td>16-26</td>
</tr>
<tr>
<td>Handedness (Right/Left)</td>
<td>10 / 12</td>
<td>10 / 12</td>
</tr>
<tr>
<td>Medication (Yes/No)</td>
<td>4 / 12</td>
<td>0 / 12</td>
</tr>
<tr>
<td>Comorbidity (Yes/No)</td>
<td>4 / 12</td>
<td>0 / 12</td>
</tr>
<tr>
<td>Family Diagnosis (Yes/No)</td>
<td>3 / 12</td>
<td>0 / 12</td>
</tr>
</tbody>
</table>

3.2.2 Stimuli

Four tasks were recorded for video presentation. The tasks were filmed with an 8.9 megapixel Sony camcorder (Nagasaki, Japan), and then edited into 30 seconds blocks using Adobe Premiere. All tasks were filmed in front of a plain, white background to ensure the stimulus presented was constricted to the hand,
and objects that were manipulated. In all video-tasks, a male and female model was used. The four video tasks are displayed in Figure 3.1, and were as follows.

3.2.2.1 **Hand-Object**: this video presented a hand picking up several different objects, an apple, a glass of juice and a mango. Only the hand and arm of the actor were visible.

3.2.2.2 **Hand-Mouth**: this video presented an actor bringing a piece of food to the mouth, and chewing. The pieces of food used were a banana and a biscuit. In this condition, the hand and face of the actor were visible.

3.2.2.3 **Hand-Communicative**: this video presented a hand performing several communicative gestures. The gestures performed were a wave, thumbs up, and an ‘OK’ gesture. Only the hand and arm of the actor was visible.

3.2.2.4 **Hand-Directive**: this video presented a hand performing several directive gestures. The gestures performed were a finger pointing, and a hand motioning to ‘stop’. Only the hand and arm of the actor was visible.

![Figure 3.1](image)

**Figure 3.1.** The four different hand actions participants observed in the fMRI: A. Hand-Object. B. Hand-Mouth. C. Hand-Directive. D. Hand-Communicative.
3.2.3 Stimulus Presentation

Participants lay flat on the bed of the scanner with their head placed within the head coil. Cushions around the head coil restricted head movement. Participants watched the videos via a mirror that was positioned above their head. Videos were projected on this mirror from an outside computer. The viewing distance for participants was approximately 55-60cm. Prior to each video, participants were instructed to carefully observe each of the four video sequences and remain as still as possible.

The video experiment was conducted with a block design, which alternated between experimental and control conditions. Each of the four video tasks was 6 minutes in length, and alternated between 30-second blocks of experimental task and control condition. The duration of each experimental or control block was 11 TRs, with the beginning of the next block being synchronised to the following TR (i.e. at TR 12, 23, 34). Consistent with past research in this field (Buccino et al., 2002; Buccino et al., 2004; Martineau et al., 2010), the control video for all tasks was a picture of a motionless hand, with the exception of the hand-mouth task, which was a picture of an expressionless face. In all four video tasks, the control condition was shown first to participants. In each trial, the subject observed 6 experimental blocks and 6 control blocks. At the end of each trial, participants were asked to verbally report on what actions they saw in the scanner. This was a purely qualitative step to ensure participants were paying attention to the stimulus.

In order to display the videos in the scanner, Presentation® (Neurobehavioural Systems) software was used. The timings of the video were
synchronized to the scans TR. A scenario file utilizing an in-house script was run through presentation to ensure that each 30-second block of video that alternated between experimental and control condition went for exactly 11 repetition times (TRs), where the next block was synchronised to begin on the proceeding TR. In total, each of the four video sequences went for 132 TRs.

### 3.2.4 fMRI Acquisition and Pre-processing

All MRI images were collected with a 3 T Siemens Tim Trio scanner (Erlangen, Germany) with a birdcage quadrature head-coil. Whole-brain BOLD weighted fMRI images were acquired using a gradient-recalled, interleaved echo-planar imaging (EPI) sequence (TR = 3.0 s; TE = 40ms; flip angle = 60°; FOV = 24 x 24 cm; 128 x 128 matrix).

All DICOM fMRI images were pre-processed using SPM8 for MAC (Institute of Neurology, University College, London, 2011) and MATLAB (2007, The MathWorks, Natick, MA, USA). Several pre-processing steps were conducted, beginning with temporal alignment of slices within each volume to the first slice, rigid-body spatial realignment to correct for subject movement, spatial normalisation into standard space, re-sampling images into isotropic voxels (2x2x2mm³), and spatial smoothing with a Guassian kernel (FWHM = 8mm). All images were normalized to the standard Montreal Neurological Institute (MNI) template image of 152 brains.
3.2.5 fMRI Image Analysis

3.2.5.1 Within Groups Analysis

This study utilized a BOLD analysis on each individual participant. In accordance with the general linear model, statistical parametric maps were generated for the following comparisons; hand-object vs. static, hand-mouth vs. static, hand-communicative vs. static, hand-directive vs. static. The four video tasks were then combined together (i.e. an SPM.mat file was generated for each participant combining each of the 4 hand tasks). This was completed in the hope it would increase sensitivity to signal change in MN regions. To look for statistically significant increases in BOLD within each group, single sample student t-tests were completed between these two conditions.

Activation maps were examined with a visualization program (xjView; http://www.alivelearn.net/xjview8/) to determine the localization and extent of cerebral activity. This program utilizes the WFU PickAtlas database, which represents cortical areas in MNI space. Several past studies have used xjView for localization of brain regions (You et al., 2011; Schunck et al., 2008). Localization of brain region was further verified by a neurosurgeon who was a member of this research team (R. Bittar).

Contrasts were performed on the whole brain using standard threshold criteria (Penny & Holmes, 2003) with a voxel threshold for statistical significance of \( p < .001 \). In order to determine whether the task activated the hypothesized areas, small volume corrections were conducted with a radius of 10mm (similarly to Calvo-Merino, Glaser, Grezes, Passingham & Haggard, 2005). The co-ordinates were selected based upon localization of anatomical
regions in xjView, and previous research (Buccino et al. 2004). The Regions of Interest (ROIs) in which small volume corrections were performed were the bilateral *pars opercularis* (BA44), the anterior part of the IPL (BA40), the PMC (BA6) and STS (roughly corresponding to BA22).

Within these areas of interest, significant activation was thresholded using cluster-wise significance (*p*<.05, FWE corrected to control for multiple comparisons). For reporting of other significant regions of activation, whole brain cluster level significance with a threshold of *p*<.05 FWE corrected was implemented. This was in accordance with guidelines for reporting fMRI studies by Poldrack et al. (2008) to control for false positives.

### 3.2.5.2 Between groups analysis

Following the within group *t*-tests, second stage random effects analyses were conducted using two sample *t*-tests. In order to thoroughly explore differences in activation between the groups, bi-directional comparisons were made (i.e. increased BOLD response in TD individuals and participants with HFA/AS). To test whether the groups differed in BOLD response in hypothesized regions, small volume corrections were conducted using the same criteria and co-ordinates as the within groups analyses (FWE corrected, *p*<.05). Likewise, whole brain, cluster-wise activation at *p*<.05 FWE corrected was used to report any other regions found to be significantly different between the groups.
3.3 Results

3.3.1 Within Group Analysis

For both groups, a significant BOLD increase was observed in all *a priori* specified MN regions (Table 3.2). These were pre-frontal (*pars opercularis*, PMC), parietal (IPL) and temporal (STS) MN regions. An example of increased BOLD localised to the PMC for both groups is demonstrated in Figure 3.2.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Side</th>
<th>k</th>
<th>MNI coordinates</th>
<th>Z score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Typically Developing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pars Opercularis (BA44)</td>
<td>R</td>
<td>198</td>
<td>50 12 20</td>
<td>5.13***</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>58</td>
<td>-48 14 14</td>
<td>3.58**</td>
</tr>
<tr>
<td>Premotor Cortex (BA6)</td>
<td>R</td>
<td>177</td>
<td>44 4 56</td>
<td>5.31***</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>258</td>
<td>-44 2 50</td>
<td>4.87***</td>
</tr>
<tr>
<td>Inferior Parietal lobule – Supramarginal (BA40)</td>
<td>R</td>
<td>165</td>
<td>46 -36 46</td>
<td>4.26***</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>414</td>
<td>-40 -46 54</td>
<td>5.69***</td>
</tr>
<tr>
<td>Superior Temporal Sulcus (BA22)</td>
<td>R</td>
<td>515</td>
<td>52 -54 8</td>
<td>7.60***</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>515</td>
<td>-44 -54 10</td>
<td>7.06***</td>
</tr>
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<td><strong>High Functioning Autism/Asperger’s Syndrome</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pars Opercularis (BA44)</td>
<td>R</td>
<td>155</td>
<td>50 16 18</td>
<td>5.25***</td>
</tr>
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<td></td>
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<td>147</td>
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<td>4.28**</td>
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<tr>
<td>Premotor Cortex (BA6)</td>
<td>R</td>
<td>162</td>
<td>48 4 54</td>
<td>3.65**</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>241</td>
<td>-44 0 56</td>
<td>5.48***</td>
</tr>
<tr>
<td>Inferior Parietal lobule – Supramarginal (BA40)</td>
<td>R</td>
<td>101</td>
<td>46 -36 46</td>
<td>4.96***</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>462</td>
<td>-38 -42 50</td>
<td>5.47***</td>
</tr>
<tr>
<td>Superior Temporal Sulcus (BA22)</td>
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<td>515</td>
<td>52 -46 8</td>
<td>7.31***</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>467</td>
<td>-54 -48 8</td>
<td>6.57***</td>
</tr>
</tbody>
</table>

Voxel threshold set at *p* < .001 uncorrected after random effects analysis. Significance was evaluated using small volume corrections on group statistic parametric maps with a spherical radius of 10mm. Significance was set at *p* < .05, FWE corrected. The regions in the table above were hypothesized to be part of the mirror neuron system, based upon coordinates of previous research (Buccino et al., 2002; Calvo-Merino et al., 2005).

k = number of voxels per cluster, x, y and z = mediolateral, anterposterior and dorsoventral coordinates respectively in the MNI average brain, Z score = peak Z score in cluster. * = *p* < .05, ** = *p* < .01, *** = *p* < .001.
Figure 3.2. Activation maps depicting BA6 (PMC) for TD participants (left) and individuals with HFA/AS (right). The yellow regions correspond to BA6, whilst the red regions depict observed activation whilst participants observed goal directed hand actions. The activation in both groups roughly corresponds to Area F5, which past research has identified responds to the observation of hand and hand-mouth actions (Cattaneo & Rizzolatti, 2009).

During the hand observation task, several other voxel clusters were activated for both groups. Peak clusters and their corresponding MNI coordinates in the brain are reported for TD and HFA/AS individuals in Table 3.3. Both groups demonstrated a similar pattern of activation, corresponding to visual regions known to respond to observation of movement (middle occipital gyrus), and frontal regions known to have a role in higher-order cognitive processes (inferior and middle frontal gyrus).

However there were some notable differences in activation between the groups. Participants with HFA/AS demonstrated additional significant clusters in temporal (inferior and middle temporal gyrus) parietal (inferior), frontal (operculum), and cerebellar (posterior) areas. In contrast, TD participants demonstrated additional peaks in medial temporal regions (parahippocampus and hippocampus), the basal ganglia (putamen, lentiform nucleus) and midline structures (thalamus).
Voxel threshold set at \( p < .001 \) uncorrected after random effects analysis. Clusters reported significant at \( p < .05 \), FWE corrected.

\( k \) = number of voxels per cluster, \( x \), \( y \) and \( z \) = mediolateral, anterposterior and dorsoventral respectively in the MNI average brain, \( Z \) score = peak \( Z \) score in cluster. * \( p < .05 \), ** \( p < .01 \), *** \( p < .001 \)

### 3.3.2 Between Group Analysis

The results of the between groups analysis are summarized in Table 3.4.

In regard to hypothesized regions, only one difference was identified. The HFA/AS group demonstrated a significantly greater BOLD signal in a small cluster located in BA6, corresponding to the right PMC, bordering the precentral gyrus (Figure 3.3). No hypothesized regions were identified to be more active in
TD participants compared to participants in the HFA/AS group. In addition, several other regions were found to be different between the groups. Increased BOLD signal was noted in the right hemisphere of TD participants in visual (cuneus and calcarine gyrus) and temporal (middle temporal gyrus) regions, whilst increased BOLD was noted among individuals with HFA/AS in two right hemisphere frontal regions (the ACC and medial frontal gyrus. These results are depicted in Figure 3.4.

Table 3.4  Summary of regions that were significantly different between individuals with HFA/AS and TD participants. For both groups, mirror neuron and other significant regions that were different between the groups are reported. Negative Z-scores indicate HFA/AS had greater activity than did TD subjects, while positive Z-scores indicate TD had greater activity than HFA/AS subjects.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Side</th>
<th>k</th>
<th>MNI coordinates</th>
<th>z score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirror Neuron Regions</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pars Opercularis</td>
<td>R or L</td>
<td></td>
<td>NSV</td>
<td></td>
</tr>
<tr>
<td>Premotor Cortex (BA6)</td>
<td>R</td>
<td>34</td>
<td>34 -10 56</td>
<td>-3.96*</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>NSV</td>
<td></td>
</tr>
<tr>
<td>Inferior Parietal Lobule (BA40)</td>
<td>R or L</td>
<td></td>
<td>NSV</td>
<td></td>
</tr>
<tr>
<td>Superior Temporal Sulcus</td>
<td>R or L</td>
<td></td>
<td>NSV</td>
<td></td>
</tr>
<tr>
<td>Other significant regions</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Calcarine</td>
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<td>4.07*</td>
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<td>Cuneus</td>
<td>R</td>
<td>296</td>
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<td>3.87*</td>
</tr>
<tr>
<td>Middle Temporal Gyrus</td>
<td>R</td>
<td>296</td>
<td>34 -60 10</td>
<td>3.57*</td>
</tr>
<tr>
<td>Medial Frontal Gyrus</td>
<td>R</td>
<td>641</td>
<td>12 38 -12</td>
<td>-4.23***</td>
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<tr>
<td>Anterior Cingulate (BA24)</td>
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<td>12 26 -4</td>
<td>-4.09***</td>
</tr>
</tbody>
</table>

Voxel threshold set at $p<.001$ after random effects analysis. For mirror neuron regions, small volume corrections with spherical radius of 10mm were performed. Significance was evaluated at $p<.05$ FWE corrected. For other significant regions, whole brain analysis was performed with significance evaluated at $p<.05$ FWE corrected.

$k =$ number of voxels per cluster, $x$, $y$ and $z =$ mediolateral, anterposterior and dorsoventral respectively in the MNI average brain, Z score = peak Z score in cluster, NSV = No Significant Voxels. * $p<.05$, ** $p<.01$, ***$p<.001$. 
Figure 3.3. Results of between group comparisons where BOLD signal of TD participants was contrasted with individuals with HFA/AS. Increased BOLD signal of individuals with HFA/AS compared to TD individuals is shown in red, and can be seen in the right premotor cortex (topography of BA6 shown in yellow). The activation in the left hemisphere, which can be seen in the dorsoventral plane, borders the medial frontal gyrus and BA6.

Figure 3.4. Difference in activation between the two groups superimposed onto a single brain from random effects analysis. Scan images move from inferior (top left) to superior (bottom right). Activity from TD participants is shown in red, whilst activity from HFA/AS participants is shown in blue. The majority of activation that was increased in TD participants was in the Occipital/Temporal Lobes. In contrast, the HFA/AS group demonstrated increased activation in the frontal lobes. This figure shows the distinct patterns of activation whilst observing the hand tasks for the two groups. Clusters that remained significant when controlling for multiple comparisons are summarised in Table 3.2.
3.4 Discussion

It is relatively well established that in TD individuals, a fronto-parietal network believed to possess MNs is activated by the observation of purposeful biological movement (Rizzolatti & Craighero, 2004). Past research using fMRI provides some evidence for an increased BOLD response in frontal mirror regions among those with an ASD when observing hand actions (Martineau et al., 2010; Williams et al., 2006), but an equal number of studies reports no difference to TD individuals (Dinstein et al., 2010; Marsh & Hamilton, 2011). Thus, the present study aimed to further assess the brain response of participants with autism during observation of hand actions.

3.4.1 Hypothesized Findings

The first hypothesis that both groups would demonstrate a significant BOLD response in MN regions during action observation was supported. The within group analysis demonstrated activation in all hypothesized areas, constituting frontal (PMC, pars opercularis), parietal (IPL) and temporal (STS) MN regions. Activation in these brain regions whilst observing hand-based gestures supports a large number of past neuroimaging studies (Buccino et al., 2002; Buccino et al., 2004; Manthey Schubotz & Cramon, 2003; Molnar-Szakacs et al., 2008). Similarly to Buccino et al. (2002), activity in BA6 (see Figure 3.2), and a cluster in BA44 that extended into BA45 was observed in both groups. Signal peaks were observed in the IPL of both groups, which is known to be active in response to object-directed hand actions (Grezes et al., 2003). Finally,
The STS is known to respond to the observation of biological motion (Aziz-Zadeh et al., 2006) and tasks requiring social attention (Redcay, 2008), and was active in both groups.

The second hypothesis that participants with HFA/AS would demonstrate an increased BOLD response in MN regions was partially supported. The between groups analysis revealed a significantly greater BOLD response in the right PMC of the HFA/AS group compared to TD participants. Although difficult to quantify precisely, this cluster appeared to form part of the dorsal PMC. This region is estimated to begin at coordinate z=51 (Rizzolatti, Fogassi & Gallese, 2002), with overlap with the ventral PMC occurring between z=30-46 (Mayaka, Corcos, Leurgans & Vaillancourt, 2006). In the present study, the PMC cluster which demonstrated increased BOLD in the HFA/AS group had a local maxima at z=56 (Table 3.4), making it clearly dorsal by the criteria of Rizzolatti et al. (2002).

The dorsal PMC is considered part of the extended MN system (Keysers et al., 2013), and is believed to have a functional role in imitation (Caspers et al., 2010). It must be acknowledged however that this particular study had a relatively long TE (40ms). This can generate more inhomogeneities in the IFG (McCarthty, Blamire, Rothman, Gruetter & Shulman, 1993), generating stronger signal in dorsal relative to ventral regions. Thus, the more dorsal activation may be partially attributable to methodological factors.

Using fMRI, to date only Williams et al. (2006) has identified premotor abnormalities in participants with an ASD during an imitation paradigm. However, previous EEG research provides indirect evidence of premotor anomalies, with ASD participants exhibiting reduced suppression of the mu
rhythm during observation of hand actions compared to TD participants (Martineau et al., 2008; Oberman et al. 2004; Oberman et al., 2008). Recent evidence suggests that \textit{mu} suppression co-varies with BOLD activation in the PMC (Arnstein, et al., 2011), which may indicate that \textit{mu} suppression is attributable to inhibitory activation in the PMC. Taking these fMRI and EEG findings together, it could be speculated that premotor anomalies in autism are characterized by impaired inhibition, and excessive excitation.

Supporting this view, there is some evidence emerging from TMS research that those with an ASD demonstrate faulty inhibitory mechanisms in motor areas (Enticott, Rinehart, Tonge, Bradshaw & Fitzgerald, 2012). This is further strengthened by findings of reduced GABA\textsubscript{A} in post-mortem autistic brains (Collins et al., 2006; Fatemi et al., 2009). However, more cognitive research is necessary to clarify the functional significance of premotor abnormalities. Schutbotz and Cramon (2003) point out that it remains unclear whether premotor activity during mirror tasks reflects neural coding for goal-specific actions, or a generalized response to object movement.

Both Dinstein et al. (2010) and Marsh and Hamilton (2011) report no difference between ASD and TD groups in frontal and parietal MN regions. As mentioned however, the contrast of conditions in these studies differed to the present study, and that of other past studies (i.e. Martineau et al., 2010; Williams et al., 2006). Although at this point in time there is not yet consensus regarding how to measure MN regions with fMRI, it is not surprising that different paradigms produce different results. Perhaps in future, a more rigorous control condition could implement a non-moving hand to keep the two conditions
visually equivalent, but also have the entire image moving around the display to cancel out the influence of non-biological movement.

Compared to the present study, both Dinstein et al. (2010) and Marsh and Hamilton (2011) utilized an fMRI analysis that has been argued to be more sensitive to measuring MNs. Known as suppression repetition, the logic behind this technique is that many neuron types demonstrate a diminished response to repetition of the same stimuli (Keysers et al., 2013). Thus any region that demonstrates this property of suppression in response to execution and observation of a movement is part of the MN system. Proponents of this method would argue an advantage of these studies over the present one is that they were able to link action observation with execution, a key property of MNs (Rizzolatti & Craighero, 2004).

However what is less well established is whether MNs actually possess the property of stimulus-induced suppression. Research on monkeys has demonstrated MNs do not possess this characteristic (Caggiano et al., 2013; Keysers et al., 2003), which raises the possibility that repetition suppression could produce false-negative results. Further, recent data refutes the original claim of this technique that it allows for specific neural populations to be imaged (Bartels, Logothetis & Moutoussis, 2008).

Nevertheless, the means by which MN activity was quantified in the present study is limited. Keysers et al. (2013) recommend that a region should only be considered putatively mirror related if it demonstrates activation during both observation and execution. In the present study, only action observation was tested, meaning the analyses in this study were less sensitive to the matching mechanism that characterizes MNs. By extension, the present study was also less
able to discern MN activity from other cortical processes that may occur in tandem. However, this issue is somewhat inherent to all fMRI studies. BOLD is not particularly sensitive at distinguishing different neuron classes, which is problematic when considering MNs are estimated to constitute only 5-33% of neurons in a cortical region (Mukamel et al., 2010).

Given that the ROIs examined in this study were specified *a priori*, and based upon past MN research (i.e. Buccino et al., 2001; Buccino et al., 2004); it is argued that the observed changes in BOLD at least overlap with MN areas. However, future research utilizing more discerning fMRI measures such as pattern classification (Etzel, Gazzola & Keysers, 2008) will help refine knowledge of (a) the distribution of MNs in humans, and (b) their role in ASD.

Another limitation of the present study stems from pooling the four conditions together. Although one benefit of doing this was increased sensitivity to MN regions, it also has the potential to confound the comparisons. For instance, differences in participants head motion could produce low frequency drift in the scanner signal. Although studies have been done which suggest motion and physiological noise are not responsible for low frequency drift (Smith et al., 1999), modelling a covariate of no interest may have addressed this issue.

Given the developmental nature of autism, age-related changes are also an important area for future research. One recent study reported that activation of the inferior frontal gyrus increases with age in participants with autism (Bastiaansen et al., 2011), whilst Oberman et al. (2013) has reported that both TD and ASD participants demonstrate age related increases in *mu* suppression. These findings of age related changes may also contribute to mixed findings in the literature, when considering the cohort of Marsh and Hamilton (2011) had a
mean age of 33, by comparison to the present study that was 19. Unfortunately the sample size in this study was too small to look at meaningful age related
differences, but it is possible a younger sample may have generated different results.

This study provided no evidence of differences in activation of the IPL, BA44 or STS between the two groups. Most previous fMRI investigations of MNs in autism report no differences in the parietal component of the mirror system (Dinstein et al., 2010; Marsh & Hamilton, 2011; Martineau et al., 2010), which is interesting given its functional importance to intention understanding (Hamilton & Grafton, 2006; Tunik et al., 2007). Thus, the present result is unlikely to represent a global deficit in the MN network. However, given previous research has reported structural deficits in the IPL and STS (Hadjikhani, Joseph, Snyder & Tager-Flusberg, 2006); it remains possible that different anomalies occur in these areas, which were not assessed by the present study.

3.4.2 Non-Hypothesized Findings

Looking at this study more generally, individuals with HFA/AS demonstrated a general pattern of greater frontal activity than TD participants. Frontal lobe anomalies are among the most commonly identified in ASD, and include neuro-inflammation (Vargas, Nascimbene, Krishnan, Zimmerman & Pardo, 2005) brain size abnormalities (Carper & Courchesne, 2005), anomalous interactions between microglia and neurons (Morgan et al., 2012) and increased minicolumnar density (Casanova et al., 2006). Furthermore, Courchesne and Pierce (2005) note that frontal lobe differences between those with an ASD and
TD participants are identified in almost all fMRI investigations, encompassing tasks such as memory, attention, embedded figures and language.

The specific frontal region to demonstrate increased BOLD in the HFA/AS group was a cluster spanning the rostral ACC and medial frontal gyrus (Table 3.3). These two regions are believed to have a role in executive functioning (Talati & Hirsch, 2006), with the ACC linked to poorer inhibition of responses (Agam, Joseph, Barton & Manoach, 2010), learning (Bush, Luu & Posner, 2000) and error-detection (Bush et al., 2002). Moreover, an activation likelihood estimation meta-analysis of 15 fMRI studies by Di Martino et al. (2009) revealed that during social tasks, the rostral ACC, along with the supplementary motor area is the most common region in autism to be identified as hyper-active.

Recently, the ACC has also been linked to MN functioning. The only single cell study to have measured MNs in humans, reported that 17% of neurons measured in the rostral ACC discharged in response to action observation. This result did not reach significance however. Furthermore, in a meta-analysis of 125 fMRI MN studies, the right ACC was one of 14 significant clusters to be attributed to MN functioning in humans (Molenberghs et al., 2011). Although based on limited evidence, it is possible an extended model of the MN network could include the ACC.

Interestingly, although Marsh and Hamilton (2011) report no differences between TD and ASD participants in the fronto-parietal network, they found that TD participants activated the mid-cingulate during action observation, where this activity was absent in participants with an ASD. Although this finding is in the
opposite direction to the present study, it raises the possibility that the cingulate cortex is an important region of difference in studies of MN function in autism.

TD individuals demonstrated stronger occipital activity than was seen in those with HFA/AS, in regions including the calcarine gyrus and cuneus. The middle temporal gyrus was also found to be more active in TD participants. Reduced occipital and temporal activation in those with HFA/AS would appear to suggest a difference in visually based processing of hand movements. Surprisingly however, there are very few studies to have investigated the functional organization of the visual system in individuals with an ASD. Two cognitively based studies suggest those with autism are less sensitive to visual motion (Blake, Turner, Smoski, Pozdol & Stone, 2003; Gepner & Mestre, 2002).

As the present study did not assess cognitive differences between the groups, explaining the pattern of increased frontal activity in ASD and increased visual activity in TD participants remains speculative. One possibility is that the increased frontal activation may reflect ‘cortical inefficiency’, where ASD participants possess a critical dependence on magnitude of activation when directing attention toward a stimulus (Karlgodt et al., 2007). However, this has been observed in disorders such as schizophrenia (Manoach, 2003), making it an inadequate explanation of the features of autism exclusively. Other potential explanations for the pattern of increased frontal and decreased occipital activation in autism include weakened central coherence (Behrmann, Thomas & Humphries, 2006), executive function anomalies (Ozonoff, Pennington & Rogers, 1991), and differences in focus of visual attention (Klin, Jones, Schultz, Volkmar & Cohen, 2002).
3.4.3 Conclusion

In sum, the present study identified that individuals with HFA/AS possessed increased BOLD in the right, dorsal PMC, which may be attributable to MNs. This contributes to a small research base that has looked specifically at hand actions, and identified increased BOLD in frontal MN regions (Martineau et al., 2010; Williams et al., 2006). It further adds to the broader literature identifying pronounced frontal disturbance in individuals with an ASD (Courchesne and Pierce, 2005). Similarly to recent investigations, no difference was observed between the groups in parietal or temporal mirror areas (Dinstein et al., 2010; Marsh & Hamilton, 2011; Martineau et al., 2010). Increased BOLD in participants with HFA/AS was also identified in the ACC, which may be linked to MN functioning.
Chapter 4: Functional connectivity of the mirror neuron system in participants with autism
4.1 Introduction

Increasingly, Autism Spectrum Disorders (ASD) are being recognized as conditions that impact neural networks, moreso than localised, regional brain disturbances (Vissers, Cohen, & Geurts, 2012). Neurodevelopmental abnormalities shortly after birth in cortical layering (Fatemi, Stary, Halt, & Realmuto, 2001), minicolumnar width (Casanova et al., 2006) and overall growth (Courchesne et al., 2001; Courchesne, Carper & Akshoomoff, 2003; Dawson, et al., 2007; Schumann et al., 2010) may alter the development of experience dependent neural networks, which in turn, impairs cortical organization (Rudie et al., 2011). On this basis, research has begun to investigate anomalies in both functional and structural brain connectivity in ASD.

Several early studies using Positron Emission Tomography (PET) found that ASD are associated with reduced correlations in activity between cortical regions (Horwitz, Rumsey, Grady & Rapoport, 1988; Castell, Frith, Happe & Frith, 2002). These findings have since been elaborated on by directly measuring Functional Connectivity (FC); a functional magnetic resonance imaging (fMRI) analysis that measures low frequency, interregional correlations in the brain. Although FC does not establish causal relationships between brain regions, it can quantify the degree of synchronisation between areas (Sporns, Tononi & Edelman, 2000).

There is mounting evidence that participants with ASD possess abnormal FC compared to typically developing (TD) individuals (Vissers et al., 2012). At present, the most prominent theory is that those with ASD possess overall reduced FC (Just, Keller, Malava, Kana & Varma, 2012). The majority of
evidence for reduced FC in autism has come from research where participants engage in specific cognitive and affective tasks. There is evidence of reduced FC in individuals with ASD during tasks that involve language (Just, Cherkassky, Keller & Minshew, 2004; Kana, Keller, Cherkassky, Minshew & Just, 2006), executive functioning (Just, Cherkassky, Keller, Kana & Minshew, 2007), memory (Koshino et al., 2005), visuomotor skills (Villa-Lobos, Mizuno, Dahl, Kemmotsu & Muller, 2005), and social skills (Koshino et al., 2008). All of these studies demonstrated reduced FC from frontal nodes to more posterior brain regions.

Although some of these studies further linked reduced FC to poorer task performance (Just et al., 2007), others found TD and ASD participants performed equivalently well (Kana et al., 2006; Koshino et al., 2005). The significance of reduced FC in individuals with an ASD during cognitively demanding tasks is unclear, but has been attributed to reduced co-ordination and organization of functional brain networks during attentionally demanding situations (Just et al., 2012).

Another form of FC analysis known as task-regression also utilizes cognitively based experimental designs, but partials out the effect of task-dependent brain activity. This is typically completed by implementing low-pass or band-pass filters under 0.1 Hz on the signal (Fair et al., 2007). Task-driven FC is believed to be constituted by high-frequency fluctuations in the signal, where low or band-pass filters partial out these effects (Muller et al., 2011). Focusing upon low-frequency signal fluctuations is believed to be a more sensitive measure of intrinsic connectivity in the brain.
Findings from task regression studies in participants with an ASD have generated mixed effects, with evidence of reduced FC (Jones et al., 2010), and increased FC from the thalamus (Mizuno, Villalobos, Davies, Dahl & Muller 2006) motor cortex (Turner, Frost, Linsenbardt, McIlroy & Muller, 2006) and extrastriatal regions (Noonan, Haist & Muller, 2009).

A third method for studying FC is during a ‘resting state’, where participants lie in the scanner and let their mind wander. Because these studies lack controlled experimental conditions, it is not possible to use task-regression to estimate and remove cognitively based signal changes (Muller et al., 2011). Nevertheless, band-pass filtering can still be used to minimize the impact of such fluctuations in the blood oxygen level dependent (BOLD) signal. These studies have typically investigated the default mode network, which is a baseline or ‘idling’ state of brain activity that is observed when a subject is not attending to the outside world (De Luca, Beckmann, De Stefano, Matthews & Smith, 2006; Fox & Greicius., 2010). However, resting-state scans are not limited to studying the default mode network (Anderson et al., 2011).

Similarly to task-regression paradigms, resting-state FC has generated mixed effects. A number of resting state studies report reduced FC in participants with an ASD (Anderson et al., 2011; Assaf et al., 2010; Kennedy & Courchesne, 2008; Weng et al., 2010), whilst other studies have reported a mix of increased and decreased FC (Monk et al., 2009; Maximo, Keown, Nair & Muller, 2013; Paakki et al., 2010), and no difference to TD participants (Tyszka, Kennedy, Paul & Adolphs, 2013).

Based upon the above research, it is clear FC research into ASD has produced inconsistent findings. One contributing reason for these mixed findings
is that there is no clear consensus regarding methodology in the literature. Studies have varied in regard to (a) low pass versus high pass filtering, (b) task activation versus task regression versus resting-state scans, and (c) whole-brain versus network-specific designs. In a meta-analysis of 32 FC studies, Muller et al. (2011) report that all past studies that have not used low or band-pass filters have identified reduced FC in ASD. On the other hand, all studies to have combined low-pass filtering, task regression and whole-brain analyses report mixed effects, including greater FC in participants with ASD. Thus, differences in methodology and analysis appear to have a direct impact upon findings, meaning research needs to be interpreted within this context.

Furthermore, there are theoretical issues to consider when examining FC. For example, it remains unclear precisely what increased FC in clinical populations may denote. Although FC is agreed to reflect a measure of coordination between two or more brain regions (Just et al., 2012), over-connectivity or additional network activation in individuals with an ASD could potentially reflect inefficient, non-parsimonious brain processes, or noisier activation (Vissers et al., 2012). Finally, more research is required to test specific, a priori networks. It is possible that particular patterns of difference in FC between ASD and TD samples could depend upon the network measured, or region seeded.

A candidate network to examine FC in participants with autism is the mirror neuron (MN) system (Gallese & Sinigaglia, 2011; Rizzolatti & Craighero, 2004). The MN system is constituted by the premotor cortex (PMC), pars opercularis, convexity of the inferior parietal lobule (IPL) and superior temporal sulcus (STS) (Aziz-Zadeh, Koski, Zaidel, Mazziota & Iacoboni., 2006; Iacoboni
et al., 2001; Cattaneo & Rizzolatti, 2009). Although there is fMRI evidence of dysfunction in these regions in autism using standard block design paradigms (Dapretto et al., 2006; Hadjikhani et al., 2007; Martineau, Andersson, Barthelemy, Cottier, & Destrieux, 2010), little research has examined FC in these regions.

Two studies examining FC from the inferior frontal gyrus identified increased FC in individuals with an ASD to the superior frontal gyrus (Rudie et al., 2010; Shih et al., 2010); with Rudie et al. (2010) also identifying reduced FC to the supramarginal gyrus. A further task-based study investigating visuomotor co-ordination by Villa-Lobos et al. (2005) identified reduced FC from visual area BA17 to the *pars opercularis* among those with an ASD. A more recent resting-state study by Ebisch et al. (2011) investigated FC from the insula in a social cognition network overlapping with MN regions, and report reduced FC in autism participants from the somatosensory cortices and amygdala. On the basis of this small number of studies, differences in FC of the MN network in autism yield inconsistent results that parallel the broader FC literature.

Thus, the present study aims to investigate FC across the whole-brain, during a resting-state scan. The right PMC, and right anterior cingulate cortex (ACC) were selected as seed regions. The coordinates of these seed regions were selected based upon their atypical response in the HFA/AS group in Study 1. Additionally, the PMC is part of the core fronto-parietal MN network (Rizzolatti & Fabbri-Destro, 2008; Perkins, Stokes, McGillivray & Bittar., 2010; Rizzolatti & Craighero, 2004), whilst the ACC has also been linked to MN function (Mukamel et al., 2010; Ramachandran & Oberman, 2006). Finally, atypical BOLD response of the PMC (Williams et al., 2006) and ACC (Di Martino et al.,
2009) has previously been reported in ASD. On this basis, two primary hypotheses, and a third exploratory hypothesis were generated for the present study:

H1: Based upon previous MN literature, the right PMC will demonstrate significant FC with other components of the fronto-parietal MN network in both groups (pars opercularis, IPL, STS).

H2: Based upon the findings of Study 1, the right PMC seed will demonstrate significantly greater FC with other components of the fronto-parietal MN network in the HFA/AS group.

H3: Based upon the findings of Study 1, the ACC seed will demonstrate increased whole-brain FC in the HFA/AS group by comparison to the TD group.

4.2 Method

4.2.1 Participants

This study received ethics approval from the Deakin University Human Research Ethics Committee (DURHEC 135-2009). As the National Health and Medical Research Council covered this ethics approval, it also conformed to the Austin and Repatriation Medical Centre Ethics Committee, which covers fMRI scans at the Melbourne Brain Centre (Austin Health, Melbourne, Australia). The present study utilized the same cohort as Study (1), comparing 12 TD participants with 12 individuals who had been diagnosed with either High-Functioning Autism (HFA) or Asperger’s Syndrome (AS). All Participants were male, within a similar age range, and had been diagnosed by an experienced Clinical
psychologist in the autism field using DSM-IV (APA, 2000) criteria. A more detailed description of participants is provided in section 3.2.1, and in Table 3.1.

4.2.2 Resting-State Protocol

In order to assess FC, a task free, resting-state scan was conducted, in which participants were instructed to let their mind wander with their eyes closed. The lights of the scanner were switched off, and the subject further instructed not to fall asleep. This scan went for approximately 12 minutes (210 TRs). Following the scan, participants were questioned about their experience, to ensure they had not fallen asleep.

4.2.3 Regions of Interest Selection

A seed Region of Interest (ROI) based analysis was used, where a spherical ROI area was defined by a centre voxel coordinate, and a radius measured in millimetres. Between groups results from Study 1 were used to determine two precise ROIs, which demonstrated a task-related increase in response. In this previous experiment, HFA/AS participants demonstrated an increased BOLD response in the dorsal PMC, with a cluster peak situated in the right hemisphere at $30\ -10\ 56$, and the ACC, corresponding approximately to BA24, with a cluster peak in the right hemisphere situated at $12\ 26\ -4$. A 5mm radius was set around these co-ordinates to examine whole brain FC from each region.

4.2.4 fMRI Acquisition and Pre-processing

Participants lay flat on the bed of the scanner with their head placed within the head coil. Cushions around the head coil restricted head movement.
All MRI images were conducted in a 3 T Siemens Tim Trio scanner (Erlangen, Germany) with a birdcage quadrature head-coil. Whole-brain blood-oxygen-level-dependent (BOLD) weighted fMRI images were acquired using a gradient-recalled, interleaved echo-planar imaging (EPI) sequence (TR = 3.0 s; TE = 40 ms; flip angle = 60°; FOV = 24 x 24 cm; 128 x 128 matrix).

All DICOM fMRI images were pre-processed using SPM8 for MAC (Institute of Neurology, University College, London, 2011) and MATLAB (2007, The MathWorks, Natick, MA, USA). Several pre-processing steps were conducted including temporal alignment of slices within each volume to the first slice, rigid-body spatial realignment to correct for subject movement, spatial normalisation into standard space, re-sampling images into isotropic voxels (2x2x2mm³), and spatial smoothing with a Gaussian kernel (FWHM = 8mm). All images were normalized to the standard Montreal Neurological Institute (MNI) template image of 152 brains.

A number of further pre-processing steps were taken to tidy the data. Firstly, cerebrospinal fluid (CSF) and white matter (WM) were masked from the data. REST software comes with a number of default masks for covarying out CSF and WM. These masks are derived from default mask files included with SPM. For the present data set, these masks needed to be resized to fit (79x95x68). Following this transformation, CSF and WM were regressed from the dataset as covariates.

Secondly, movement artifacts were regressed out of the data as covariates. Motion rejection was conducted in line with Lemieux, Salek-Haddadi, Lund, Laufs and Carmichael (2007). This method utilizes Pythagoras’ theorem to estimate the magnitude of the net displacement vector (d), based upon
movements in the x y or z direction. Any total movement over 0.5mm between scans was rejected. The next three scans were also rejected, meaning four scans were rejected per movement over 0.5mm. Although more scans were rejected in the HFA/AS group, only a small number were rejected in total meaning that the total number of scans was not significantly different between the groups. Thirdly, data for all participants had the linear trend removed, and were band pass filtered (.01 → .08Hz) to reduce the impact of low-frequency drift and to exclude high-frequency impacts of noise and cognitive influences during the resting-state (Biswal et al. 1995; Masterton, Carney & Jackson, 2012).

4.2.5 fMRI Image Analysis

4.2.5.1 Within-Group Analysis

A functional connectivity analysis using 210 whole brain volumes was conducted using RESTing State fMRI Data Analysis Toolkit (REST, by Song Xiaowei, http://resting-fmri.sourceforge.net). Time-series for each subject were extracted from the ROIs examined, then correlated with every voxel in the brain to generate FC maps. This was completed individually for each subject and ROI. Each individual correlation map was converted into z-statistic maps using Fischer’s r to z transformation.

A first order random effects analyses was conducted to create FC maps for the TD and HFA/AS groups, using SPM8 for MAC. The group connectivity maps utilized standard threshold criteria (Penny & Holmes, 2003), with a voxel threshold for statistical significance of p<.001. In order to determine whether the task activated the hypothesized areas, small volume corrections were conducted
with a radius of 10mm on these regions (similarly to Calvo-Merino, Glaser, Grezes, Passingham & Haggard, 2005).

The ROIs selected were based upon previous research (Buccino, Binofski & Riggio, 2004), with co-ordinates for anatomical regions being localized with xjView (http://people.hnl.bcm.tmc.edu/cuixu/xjView). This program utilizes the WFU PickAtlas database, which represents cortical areas in MNI space. Several past studies have used xjView for localization of brain regions (You et al., 2011; Schunck et al., 2008). The ROIs in which small volume corrections were performed were the bilateral *pars opercularis* (BA44), the anterior part of the IPL (BA40), and STS (roughly corresponding to BA22). Within these areas of interest, significant activation was evaluated using cluster-wise significance (*p* < .05, FWE corrected to control for multiple comparisons).

Further, clusters and voxel peaks that survived whole-brain Family Wise Error (FWE) correction were reported, with a minimum threshold of *p* < .05. This was also done with xjView, and further verified by a neurosurgeon (Bittar 2012, author).

**4.2.5.2 Between-Group Analyses**

Two-sample t-tests were conducted to contrast differences in FC between TD and HFA/AS individuals. This analysis examined both decreased and increased FC in the HFA/AS group relative to the TD group, in accordance with the recommendations of Muller et al. (2011). These group-contrast FC maps utilized standard threshold criteria (Penny & Holmes, 2003), with a voxel threshold for statistical significance of *p* < .001. To correct for multiple
comparisons, only clusters or voxel peaks that survived Family Wise Error (FWE) correction were reported, with a threshold of $p<0.05$.

When seeding the right premotor cortex, several a priori specified ROIs were predicted to demonstrate increased FC with other parts of the MN system in the HFA/AS and TD group. As mentioned, these regions were the bilateral pars opercularis (BA44), the anterior part of the IPL (BA40) and STS (roughly corresponding to BA22). These regions were defined with xjView in the same manner as the within groups analysis.

Likewise, small volume corrections were conducted with a spherical radius of 10mm surrounding the hypothesized co-ordinates. This was done to increase sensitivity to any differences between the groups in BOLD signal for hypothesized regions only. The co-ordinates were based on localization of anatomical regions in xjView. Within these areas of interest, significant activation was evaluated using cluster-wise significance ($p<0.05$, FWE corrected to control for multiple comparisons).

4.3 Results

4.3.1 Within-group analysis

4.3.1.1 Premotor Cortex Seed

Results from seeding the right PMC demonstrated widespread FC across the brain in both groups. This activation is summarized in Table 4.1, and depicted in Figure 4.1. In both groups, FC was strongest in the middle frontal gyrus extending spatially into the superior frontal gyrus bilaterally, and the right precentral gyrus. The HFA/AS group demonstrated additional FC in the inferior
frontal gyrus, including BA9 (bilaterally), and the frontal orbitalis in the left hemisphere. The HFA/AS group also demonstrated increased FC in right inferior parietal areas, nearby the supramarginal gyrus. Most notably for the TD group was increased FC in the supplementary motor area (bilaterally), and the precuneus, that was not identified in the HFA/AS group.

Table 4.1. Co-ordinates demonstrating significant FC with the right premotor cortex seed for typically developing (TD) and High Functioning Autism/Asperger’s Syndrome (AS) participants

<table>
<thead>
<tr>
<th>Region</th>
<th>TD</th>
<th>HFA/AS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max Z</td>
<td>MNI peak (mm)</td>
</tr>
<tr>
<td>Premotor Cortex (BA6)</td>
<td>6.67***</td>
<td>30 -10 58</td>
</tr>
<tr>
<td>Cingulate Gyrus</td>
<td>5.00*</td>
<td>18 -10 44</td>
</tr>
<tr>
<td>Inferior Frontal Gyrus</td>
<td>4.95*</td>
<td>60 6 38</td>
</tr>
<tr>
<td>Inferior Frontal Gyrus</td>
<td>5.07*</td>
<td>54 2 34</td>
</tr>
<tr>
<td>Inferior Frontal Gyrus</td>
<td>5.11*</td>
<td>-52 10 32</td>
</tr>
<tr>
<td>Frontal Orbitalis</td>
<td>5.36**</td>
<td>-46 28 -4</td>
</tr>
<tr>
<td>Middle Frontal Gyrus</td>
<td>5.79***</td>
<td>40 4 42</td>
</tr>
<tr>
<td>Middle Frontal Gyrus</td>
<td>5.08*</td>
<td>-24 0 66</td>
</tr>
<tr>
<td>Prefrontal Cortex (BA9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial Frontal Gyrus</td>
<td>5.82***</td>
<td>14 -28 58</td>
</tr>
<tr>
<td>Superior Frontal Gyrus</td>
<td>5.11*</td>
<td>2 8 54</td>
</tr>
<tr>
<td>Superior Frontal Gyrus</td>
<td>5.48**</td>
<td>-6 4 70</td>
</tr>
<tr>
<td>Supplementary Motor Area</td>
<td>5.12*</td>
<td>10 2 72</td>
</tr>
<tr>
<td>Supplementary Motor Area</td>
<td>5.05*</td>
<td>-8 -8 68</td>
</tr>
<tr>
<td>Precentral Gyrus</td>
<td>6.11***</td>
<td>38 -8 54</td>
</tr>
<tr>
<td>Precentral Gyrus (BA4)</td>
<td>5.08*</td>
<td>36 -26 66</td>
</tr>
<tr>
<td>Precentral Gyrus (BA4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Central Gyrus (BA3)</td>
<td>5.18*</td>
<td>40 -26 52</td>
</tr>
<tr>
<td>Post Central Gyrus</td>
<td>5.11*</td>
<td>18 -48 70</td>
</tr>
<tr>
<td>BA2/Supramarginal</td>
<td>5.12*</td>
<td>62 -30 40</td>
</tr>
<tr>
<td>BA43/Supramarginal</td>
<td>5.17*</td>
<td>68 -20 20</td>
</tr>
<tr>
<td>Inferior Parietal Lobule</td>
<td>5.12*</td>
<td>52 -32 46</td>
</tr>
<tr>
<td>Superior Parietal Lobule</td>
<td>5.62**</td>
<td>22 -58 66</td>
</tr>
<tr>
<td>Superior Parietal Lobule</td>
<td>5.29*</td>
<td>22 -50 60</td>
</tr>
<tr>
<td>Precuneus</td>
<td>5.08*</td>
<td>-12 -50 62</td>
</tr>
<tr>
<td>Putamen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracentral Lobule</td>
<td>5.07*</td>
<td>18 -42 56</td>
</tr>
<tr>
<td>Rectus</td>
<td>5.72***</td>
<td>20 18 -14</td>
</tr>
<tr>
<td>Cingulum</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Voxel threshold set at p<.001 uncorrected after random effects analysis. Significance was set at p<.05, FWE corrected. x, y and z = mediolateral, anterposterior and dorsoventral coordinates respectively in the MNI average brain. Max Z = peak Z scores in cluster.

* = p<.05, ** = p<.01, *** = p<.001.
Figure 4.1. Whole brain FC seeded from right premotor cortex for TD (left), and HFA/AS participants (right). Slices shown for each group are 40 (transverse), 32 (sagittal), and -8 (coronal). Although a similar pattern of activation was observed for both groups, the spatial extent of this activation was greater for autism participants.

FC between the right PMC and other parts of the fronto-parietal MN network was assessed, with both groups demonstrated significant FC with all other MN regions (pars opercularis, IPL, and superior temporal sulcus). These results are summarised in Table 4.2.

Table 4.2. Co-ordinates and activation for regions hypothesized to demonstrate significant functional connectivity with BA6. Results for both TD and HFA/AS participants are displayed below

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Side</th>
<th>k</th>
<th>MNI coordinates</th>
<th>Max Z</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
<td>y</td>
</tr>
<tr>
<td>TD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pars Opercularis (BA44)</td>
<td>R</td>
<td>413</td>
<td>52</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>217</td>
<td>-52</td>
<td>8</td>
</tr>
<tr>
<td>Inferior Parietal lobule – Supramarginal (BA40)</td>
<td>R</td>
<td>102</td>
<td>-42</td>
<td>-38</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>238</td>
<td>-52</td>
<td>-40</td>
</tr>
<tr>
<td>Superior Temporal Sulcus (BA22)</td>
<td>R</td>
<td>446</td>
<td>52</td>
<td>-46</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>247</td>
<td>-46</td>
<td>-60</td>
</tr>
<tr>
<td>HFA/AS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pars Opercularis (BA44)</td>
<td>R</td>
<td>491</td>
<td>58</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>362</td>
<td>-56</td>
<td>6</td>
</tr>
<tr>
<td>Inferior Parietal lobule – Supramarginal (BA40)</td>
<td>R</td>
<td>515</td>
<td>46</td>
<td>-56</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>515</td>
<td>-54</td>
<td>-42</td>
</tr>
<tr>
<td>Superior Temporal Sulcus (BA22)</td>
<td>R</td>
<td>461</td>
<td>52</td>
<td>-48</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>484</td>
<td>-56</td>
<td>-62</td>
</tr>
</tbody>
</table>

Voxel threshold set at \( p < .001 \) uncorrected after random effects analysis. Significance was evaluated using small volume corrections on group statistic parametric maps with a spherical radius of 10mm. Significance was set at \( p < .05 \), FWE corrected. The regions in the table above were hypothesized to be part of the mirror neuron system, based upon coordinates of previous research (Buccino et al., 2002; Calvo-Merino et al., 2005).

\( k = \) number of voxels per cluster, \( x, y \) and \( z = \) mediolateral, anterposterior and dorsoventral coordinates respectively in the MNI average brain, \( Z \) score = peak \( Z \) score in cluster. * = \( p < .05 \), ** = \( p < .01 \), *** = \( p < .001 \).
4.3.1.2 Anterior Cingulate Cortex Seed

Results from seeding the right ACC demonstrated widespread FC across the brain. This activation is summarized in Table 4.3, and depicted in Figure 4.2. Activation observed in both groups was predominantly frontal (medial and superior frontal gyrus) and temporal (inferior, middle and superior temporal gyrus). The group of individuals with HFA/AS had additional activation in the caudate (bilaterally), and the left anterior cingulate cortex. Conversely, the TD group demonstrated more significant clusters in the right superior frontal gyrus, and a cluster in the right inferior frontal gyrus.

Table 4.3. Co-ordinates demonstrating significant co-activation with the right anterior cingulate cortex seed for typically developing (TD) and High Functioning Autism/Asperger’s Syndrome (AS) participants

<table>
<thead>
<tr>
<th>Region</th>
<th>Side</th>
<th>TD</th>
<th>HFA/AS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Max Z</td>
<td>MNI peak (mm)</td>
</tr>
<tr>
<td>Anterior Cingulate</td>
<td>L</td>
<td>5.51</td>
<td>-6 12 -10</td>
</tr>
<tr>
<td>Precentral Gyrus</td>
<td>R</td>
<td>6.14 60 -4 24</td>
<td></td>
</tr>
<tr>
<td>Inferior Frontal Gyrus</td>
<td>L</td>
<td>5.54 50 22 14</td>
<td></td>
</tr>
<tr>
<td>Inferior Frontal Gyrus</td>
<td>R</td>
<td>5.97 -30 24 -22</td>
<td></td>
</tr>
<tr>
<td>Medial Frontal Gyrus</td>
<td>R</td>
<td>5.68 10 62 16</td>
<td></td>
</tr>
<tr>
<td>Medial Frontal Gyrus</td>
<td>L</td>
<td>5.6 -8 42 -12</td>
<td></td>
</tr>
<tr>
<td>Medial Frontal Gyrus (BA10)</td>
<td>R</td>
<td>5.45 8 40 -8</td>
<td></td>
</tr>
<tr>
<td>Medial Frontal Gyrus (BA10)</td>
<td>L</td>
<td>5.78 -4 50 14 5.71 -6 56 -6</td>
<td></td>
</tr>
<tr>
<td>Medial Frontal Gyrus (BA11)</td>
<td>L</td>
<td>5.06 -2 30 -12</td>
<td></td>
</tr>
<tr>
<td>Middle Frontal Gyrus</td>
<td>L</td>
<td>5.1 -28 58 6 5.21 -28 32 -16</td>
<td></td>
</tr>
<tr>
<td>Superior Frontal Gyrus (BA8)</td>
<td>R</td>
<td>5.58 28 30 56</td>
<td></td>
</tr>
<tr>
<td>Superior Frontal Gyrus</td>
<td>R</td>
<td>5.14 22 62 8</td>
<td></td>
</tr>
<tr>
<td>Superior Frontal Gyrus</td>
<td>L</td>
<td>5.97 -18 60 18 5.28 -12 20 62</td>
<td></td>
</tr>
<tr>
<td>Inferior Temporal Gyrus</td>
<td>R</td>
<td>5.9 58 -12 -24 5.15 60 -8 -24</td>
<td></td>
</tr>
<tr>
<td>Middle Temporal Gyrus</td>
<td>L</td>
<td>5.57 -60 -6 -16 5.55 -60 -30 -12</td>
<td></td>
</tr>
<tr>
<td>Superior Temporal Gyrus</td>
<td>R</td>
<td>5.53 42 18 -26 5.2 52 -2 -10</td>
<td></td>
</tr>
<tr>
<td>Rectal Gyrus</td>
<td>L</td>
<td>5.4 -6 20 -22</td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>R</td>
<td>5.38 8 18 6</td>
<td></td>
</tr>
<tr>
<td>Caudate</td>
<td>L</td>
<td>5.01 -6 16 2</td>
<td></td>
</tr>
</tbody>
</table>

Voxel threshold set at $p<.001$ uncorrected after random effects analysis. Significance was set at $p<.05$, FEW corrected. x, y and z = mediolateral, anterposterior and dorsoventral coordinates respectively in the MNI average brain, Max Z = peak Z scores in cluster. $* = p<.05$, $** = p<.01$, $*** = p<.001$. 
Figure 4.2. Whole brain connectivity seeded from right Anterior Cingulate Cortex for TD (left), and HFA/AS participants (right). Slices shown for each group are -4 (transverse), 12 (sagittal), and 28 (coronal). Similarly to the PMC seed, activation with greater spatial extent was observed in the HFA/AS group.

4.3.2 Between Group Analysis

4.3.2.1 Premotor Cortex Seed

A between-groups analysis was conducted to determine if any regions demonstrated significantly different FC between HFA/AS and TD participants, using the right premotor cortex as a seed. The results of this analysis are summarized in Table 4.4. Although the within groups analysis revealed that participants in the HFA/AS group demonstrated overall greater FC, only a small number of these regions remained significant. In the HFA/AS group, the main areas of increased FC were in the left hemisphere, situated in occipital (superior occipital gyrus and cuneus) and parietal (IPL, corresponding to BA40) regions. TD participants on the other hand demonstrated a region of increased FC in a slightly posterior portion of the left insula (see Figure 4.3).
For both groups, the magnitude of the difference in FC between the PMC and IPL, and PMC and insula was quantified further (Figure 4.4). These regions were examined more closely due to their known role in the MNS. For the IPL, positive correlations in FC between the PMC and IPL was observed for both groups, with this correlation being stronger for HFA/AS participants. For the insula, a weak positive correlation with premotor cortex activity was observed for the TD group, whilst a weak negative correlation with premotor activity was observed for HFA/AS.

Table 4.4. Summary of regions that were significantly different between individuals with HFA/AS and TD participants. For both groups, mirror neuron and other significant regions that were different between the groups are reported. Negative Z-scores indicate HFA/AS had greater activity than did TD subjects, while positive Z-scores indicate TD had greater activity than HFA,AS subjects.

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Side</th>
<th>k</th>
<th>MNI coordinates</th>
<th>Z score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x y z</td>
<td></td>
</tr>
<tr>
<td>Mirror Neuron Regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pars Opercularis</td>
<td>R or L</td>
<td>84</td>
<td>-30 -10 20</td>
<td>4.83*</td>
</tr>
<tr>
<td>Inferior Parietal Lobule (BA40)</td>
<td>R</td>
<td>51</td>
<td>-46 -52 30</td>
<td>-3.96**</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>51</td>
<td>-46 -52 30</td>
<td>-3.96**</td>
</tr>
<tr>
<td>Superior Temporal Sulcus</td>
<td>R or L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other significant regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insula</td>
<td>L</td>
<td>84</td>
<td>-30 -10 20</td>
<td>4.83*</td>
</tr>
<tr>
<td>Superior Occipital Gyrus</td>
<td>L</td>
<td>395</td>
<td>-12 -96 30</td>
<td>-3.88*</td>
</tr>
<tr>
<td>Cuneus</td>
<td>L</td>
<td>395</td>
<td>0 -86 38</td>
<td>-3.91*</td>
</tr>
</tbody>
</table>

Voxel threshold set at \( p < .001 \) after random effects analysis. For mirror neuron regions, small volume corrections with spherical radius of 10mm were performed. Significance was evaluated at \( p < .05 \) FWE corrected. For other significant regions, whole brain analysis was performed with significance evaluated at \( p < .05 \) FWE corrected.

\( k \) = number of voxels per cluster, \( x, y \) and \( z \) = mediolateral, anterposterior and dorsoventral respectively in the MNI average brain, \( Z \) score = peak \( Z \) score in cluster, NSV = No Significant Voxels. * \( p < .05 \), ** \( p < .01 \), *** \( p < .001 \).
Figure 4.3. Activation maps depicting differences in FC between TD and HFA/AS participants, when seeding the right PMC. In this Figure, 9 brain slices ranging from 20 to 52 are displayed. When seeding the premotor cortex, TD participants (red) demonstrated significantly greater FC in a small cluster of voxels in the left insula. HFA/AS participants demonstrated increased FC in the inferior parietal lobule (corresponding to supramarginal gyrus), and visual regions corresponding to BA18 and 19 in the left hemisphere.

Figure 4.4. Mean Fishers Z transformed scores for TD and HFA/AS participants in the left inferior parietal lobule (IPL), and insula. For the left IPL peak, participants with HFA/AS demonstrated significantly greater FC than TD participants. Positive FC was observed for both groups, with stronger, positive FC between the right PMC and left IPL in the HFA/AS group. For the left insula peak, TD participants demonstrated significantly greater FC. However, this was characterized by weak, positive FC between the right PMC and insula in the TD group, and weak, negative FC between these two regions for participants with HFA/AS.
4.3.2.2 Anterior Cingulate Cortex

A between-groups analysis was conducted to contrast HFA/AS participants with the TD group, using the right ACC as a seed. For this analysis, no regions were significant after FWE correction. When examining FC uncorrected, a small cluster in a more medial part of the ACC and medial frontal gyrus was more active in the HFA/AS group. The TD group demonstrated greater activation in a small cluster situated in the right anterior cerebellum, and another bordering the middle occipital gyrus and middle temporal gyrus.

4.4 Discussion

The present study contrasted FC between HFA/AS and TD participants, by seeding the right PMC and ACC. These seeds were selected due to their abnormal response in the HFA/AS group in Study 1. Low frequency fluctuations were examined by band-pass filtering the signal, as this is considered a more intrinsic measure of FC in putative networks (Nir et al., 2008). FC was measured during a resting state paradigm, as this has been found to produce qualitatively similar results to task regression paradigms (Arfanakis et al., 2000; Shih et al., 2010). The over-arching finding of this study was that in the HFA/AS group, the PMC seed demonstrated a pattern of increased and decreased FC, which overlapped with regions believed to form part of the MN system. Conversely, the ACC seed yielded no significant differences in FC between the groups.
4.4.1 Right Premotor Cortex

Two primary hypotheses were generated for the right PMC. The first hypothesis, that both groups would demonstrate significant FC between the PMC seed and other components of the fronto-parietal MN system was supported. Thus, confirmation of this hypothesis provided proof of concept for functional connections between the PMC and other nodes of the MN network (pars opercularis, IPL and STS). This supports a number of previous studies to argue for the existence of a fronto-parietal network that overlaps with MN regions (Buccino et al., 2004; Iacoboni & Dapretto, 2006; Rizzolatti & Craighero, 2004).

As expected from a study of whole-brain FC, the right PMC seed generated FC which extended beyond the MN system. In both groups, FC was observed predominantly in frontal regions such as the middle and superior frontal gyrus, and parietal regions such as the inferior and superior parietal lobes. These findings are consistent with past research to have identified inferior and superior parietal regions as a bridge between vision and movement (Wise, Boussaoud, Johnson & Caminiti, 1997), that project to the dorsal PMC (Tann’e et al., 1995).

When seeding the right PMC, FC was more dispersed across the brain in the HFA/AS group. The HFA/AS group demonstrated additional FC nearby the supramarginal gyrus of the IPL, and the inferior frontal gyrus bilaterally. However, the TD group demonstrated additional FC in the supplementary motor cortex bilaterally.

The second hypothesis, that participants with HFA/AS would demonstrate increased FC in fronto-parietal MN regions was partially supported by the between groups analysis. Participants with HFA/AS demonstrated increased FC between the right PMC, and left IPL. However, no other MN regions were found
to significantly differ between the groups. This suggests stronger FC in a putative network overlapping with regions believed to form part of the MN network (Aziz-Zadeh, Koski, Zaidel, Mazziota & Iacoboni., 2006; Iacoboni et al., 2001; Cattaneo & Rizzolatti, 2009).

As no cognitive measures were taken, it remains speculative as to what the functional significance of increased FC between the PMC and IPL might be. Rushworth, Johansen-Berg, Göbel and Devlin (2004) argue that tandem activation between the PMC and IPL in the left hemisphere may underlie motor selection and intentionality, a function in which MNs have been linked to in monkeys (Fogassi et al., 2005) and humans (Iacoboni et al., 2005). Further, the left IPL is believed to have a role in gestural imitation (Mühlau et al., 2004) – another function that has been attributed to the MN network (Binkofski & Buccino, 2006; Iacoboni & Dapretto, 2006). Given these functions of the left IPL, and previous evidence of over-activation in these regions amongst ASD participants (i.e. Williams et al., 2006), it is possible the present findings may be linked to a rudimentary deficit in imitation and intention understanding in ASD.

In TD participants, FC between the right PMC and left insula was found to be significantly greater than the HFA/AS group (see Figure 4.4). However this finding needs to be interpreted with caution. The relationship observed between premotor and insula activation was so small in TD individuals, that this insula co-ordinate did not demonstrate significant FC in the within group analysis. Furthermore, in the HFA/AS group, the relationship between PMC and insula activation was negative. Thus, although a significant difference exists between the groups, the magnitude of the correlation was negligibly small in TD individuals, and negative in the HFA/AS group. Scholvinck et al. (2010) suggest
care is required when interpreting anti-correlations as they may reflect methodological artifacts, which can be a consequence of focusing upon low frequency signals.

It remains possible that this finding has functional significance. Rizzolatti and Craighero (2005) have argued individuals with autism may possess impairment in the link between motor and visual aspects of emotion, stemming from insula anomalies. The anterior insula is purported to be a bridge between the MN network and limbic system (Cattaneo & Rizzolatti, 2009; Iacoboni & Lenzi, 2002), and demonstrates an increased BOLD response when observing empathic stimuli (Carr et al., 2003). Rizzolatti and Craighero (2005) theorize that perhaps the insula is involved in matching visual aspects of emotion (i.e. disgust; Wicker et al., 2003) with its visceral motor components in the PMC. This finding contributes to research that reduced MN response in ASD may be limited to networks involved in processing emotion (Hamilton, 2013).

Looking at the results from seeding the PMC more generally, a pattern of both increased and decreased FC in participants with HFA/AS was observed. A mixed pattern of FC in participants with autism is consistent with all prior research to have utilized a similar methodology to the present study (i.e. low pass filtering and whole-brain analyses, as reviewed by Muller et al., 2011). It is also consistent with a number of past resting state paradigms to have demonstrated a mixed pattern of FC (Monk et al., 2009; Noonan et al., 2008; Paakki et al., 2010), and FC research investigating MN circuitry (Rudie et al., 2010; Shih et al., 2010).

Interestingly, all regions to demonstrate significantly different FC between the two groups were contralateral to PMC seed (see Table 4.4). At present, interhemispheric differences in FC among participants with an ASD
remains relatively unexplored. One previous study by Anderson et al. (2011) utilized a resting-state paradigm to specifically examine inter-hemispheric FC, and report the autism group possessed reduced interhemispheric FC between sensorimotor cortex, anterior insula, fusiform gyrus, superior temporal gyrus and superior parietal lobule. Thus, co-ordination of FC between the two hemispheres may be an important area of inquiry for future research.

4.4.2 Right Anterior Cingulate Cortex

The exploratory hypothesis that participants with HFA/AS would demonstrate increased FC when seeding the ACC for both groups was not supported. Although a small number of regions were found to possess both increased and decreased FC, none of these were significant in the between groups comparison following adjustment for multiple comparisons.

Nevertheless, a broad network of regions was found to co-activate with the ACC in both groups. Similarly to the PMC, co-activation was more dispersed across the brain in the HFA/AS group. Both groups activated similar temporal regions including the inferior, middle and superior temporal gyrus. However, different patterns of frontal activation were observed. The HFA/AS group demonstrated left frontal activation that was not present in the TD group (inferior, BA10 and BA11), whilst only the TD group demonstrated activation in the superior frontal gyrus. Proximal frontal co-activation with the ACC is consistent with more detailed studies of FC in the ACC (Kelly et al., 2009).

To the author’s knowledge, no study to date has investigated whole brain FC in autism, seeding the ACC. Cherkassky et al. (2006) examined interconnections between the ACC and posterior cingulate cortex and precuneus, and
report reduced FC in the autism group. However, the fact Cherkassky et al. (2006) examined ROI pairs may account for the different findings. Although there is ample evidence for functional anomalies in the ACC in autism (Agam, Joseph, Barton & Manoach, 2010; Bush et al., 2002; Bush, Luu & Posner, 2000; Ramachandran & Oberman, 2006; Talati & Hirsch, 2006), more research is required to determine if there are deficits in FC from this region.

4.4.3 Final Considerations

There are a number of further issues that warrant discussion. Firstly, the co-ordinates that were seeded were selected based upon their significance in Study 1. Selecting seed based on prior findings is common practice in FC studies (Muller et al., 2011); however, Kriegeskorte, Simmons, Bellgowan and Baker (2009) points out that regions which strongly activate together in a BOLD study may trivially be highly correlated with one another in FC studies. Furthermore, resting state fMRI cannot entirely remove cognitive based fluctuations, as it somewhat paradoxically is a highly active state. Although band-pass filters under 0.1Hz isolate and accentuate fluctuations considered to reflect network specific intrinsic FC (Muller et al., 2011; Cordes et al., 2001); task regression may be a more appropriate technique in future.

There are a number of methodological limitations that further warrant discussion. Firstly, it also must be acknowledged that the use of a priori masks is not sensitive to difference between individual scans. For instance, it is highly likely there will be individual differences in ventricle sizes, and perhaps even greater group differences between TD and HFA/AS participants. It is possible this may have biased the present findings, as covariates of CSF and WM were
crudely defined. Further, the use of three dimensional spheres to define seeds and ROIs was also crude. Although the WFU PickAtlas database in xjView was utilized to select co-ordinates, by using spheres, it is likely voxels outside of the ROIs were also subsumed in the analyses.

Interpreting precisely what increased FC in autism denotes is speculative, and must consider aspects of design and methodology. Just et al. (2012) suggests that stronger FC between regions reflects increased synchrony in activity that is orientated toward a psychological task. However, this interpretation may not apply to FC during a resting state, as is the case in this study, or to clinical populations. A more general view of FC is that it represents a history of regional co-activation, and hebbian effects of plastic changes in functional networks (Lewis, Baldassarre, Committeri, Romani & Corbetta, 2009). Based upon this view, it has been theorized that over-connectivity from frontal nodes in individuals with an ASD may interfere with normal neural interactions (Ben-Bashat et al., 2007; Shih et al., 2010).

Thus, the stronger FC in individuals with HFA/AS that was observed in the present study in fronto-parietal nodes, may point to a history of inefficient, less selective hebbian plasticity in regions overlapping with the MN network. The idea of ‘cortical inefficiency’ has been applied to other disorders such as schizophrenia (Manoach, 2003), and has been argued to potentially be an intrinsic component of heterogeneous disorders such as autism (Dichter, Felder & Bodfish, 2009). It is also of note that reduced FC has previously been found to correlate with increasing age in individuals with an ASD (Lee et al., 2009). Given the mean age of 19.83 for participants with HFA/AS in this study, it is possible that increased FC is partly attributable to a sample of young adults.
To conclude, when seeding the right PMC, differences in FC were observed between TD and HFA/AS participants. Participants in the HFA/AS group demonstrated overall greater FC in occipital (middle occipital gyrus, cuneus) and parietal (IPL) areas, whilst TD participants demonstrated greater FC in a medial temporal region, corresponding to the insula. These results may have relevance to the MN system. In particular, increased FC among those with autism in the IPL may reflect a history of cortical inefficiency in this network. Although there is ample evidence that the ACC possesses functional anomalies in autism, this study found no evidence of differences in FC when seeding this region.
Chapter 5: Structural connectivity of the mirror neuron system in participants with autism
5.1 Introduction

Recent research into ASD has placed increased emphasis upon the relevance of neural networks to its pathology, paralleling the view that complex cognitive functions arise from multi-focal networks more so than distinct, specialized anatomical regions (Mesulam, 1990). In Study 2, Functional connectivity (FC) was examined, and identified the autism group possessed increased FC between the right premotor cortex (PMC), and a number of left hemisphere regions including the inferior parietal lobule (IPL). However, an inherent caveat to FC findings is that they do not probe the underlying structure of cortical connectivity, and the organization of white matter (WM) tissue (Travers et al., 2012).

Overcoming this limitation, Diffusion Tensor Imaging (DTI) is a non-invasive technique that can identify differences in microstructural and macroscopic organization of WM (Lange et al., 2010). In WM bundles, the membranes of axons and myelin cause the diffusion of water perpendicular to WM tracts (radial diffusivity) to decrease relative to directions parallel to WM (axial), leading to anisotropic water flow (Lee et al., 2007). In DTI, this flow is represented by one axial eigenvalue ($\lambda_1$), and two radial eigenvalues ($\lambda_2, \lambda_3$), which reflect the directionality of each eigenvector (Travers et al., 2012).

On this basis, several DTI measures can be extracted. Fractional Anisotropy (FA) is a normalized value ranging from 0 to 1, which represents the fraction of the tensor that can be assigned to anisotropic diffusion (Jones, 2008). It is believed to be sensitive to structural differences in myelination, axonal density, axonal caliber and fiber coherence (Assaf & Pasternak, 2008; Cheng et
al., 2010). Two additional measures are Mean Diffusivity (MD), which represents the average radius of the three eigenvalues, and is sensitive to the density of tissue barriers in all directions, and Volume Ratio (VR) which is sensitive to regions intersecting low and high anisotropy (Bihan et al., 2001).

Examining the eigenvalues on an individual basis is argued to provide a more complete picture of WM structure (Song et al., 2005). Axial Diffusivity (AD) looks at water diffusivity parallel to WM tracts ($\lambda_1$) and is believed to be sensitive to axonal injury (Travers et al., 2012), whilst Radial Diffusivity (RD) expresses water diffusivity perpendicular to tracts ($[\lambda_2+\lambda_3]/2$) and is believed to be sensitive to dysmyelination and demyelination (Harsan et al., 2006).

DTI allows for the properties of specific WM pathways to be investigated (Alexander, Hasan, Lazar, Tsuruda & Parker, 2001). One such structure with relevance to ASD is the Superior Longitudinal Fasciculus (SLF), which is constituted by horizontal fibers in the superior parietal lobe (SLF I), angular gyrus (SLF II), and supramarginal gyrus (SLF III; Bernal & Altman, 2010). A fourth sub-division known as the arcuate fasciculus constitutes the inferior portion of the SLF, and extends further from the temperoparietal junction, arching around the sylvian fissure (Catani, Jones & Ffitche, 2005). The SLF connects the aforementioned temporal and parietal areas to the ventral premotor cortex (BA6), pars opercularis (BA44), pars triangularis (BA45) and middle frontal gyrus (BA9) (Kaplan et al., 2010; Riling et al., 2008).

Among several roles including multisensory association (Makris et al., 2005) and language (Travers et al., 2012), the SLF has been theorized to be an important structure linking the fronto-parietal mirror neuron (MN) system (Aziz-Zadeh et al., 2006). Specifically, the SLF II and III (Barnea-Goraly, Lotspeich
and Reiss, 2010) and arcuate fasciculus (Dapretto et al., 2006) are argued to be partly constituted by axons of parietal MNs. Given that a growing number of functional studies reveal anomalous activation of MN regions in autism (see Perkins, McGillivray, Bittar & Stokes, 2010 for a review), WM integrity of the SLF in ASD is a topic of interest.

At present, evidence for WM deficits in the SLF of individuals with an ASD has been inconsistent. Concerning FA, the most common finding across a broad range of age-groups is reduced FA in participants with ASD (Bakhtiari et al., 2012; Barnea Goraly et al., 2010; Bloemen et al., 2010; Jou et al., 2010; Lee et al., 2007, Shukla et al., 2010), which has been further validated by a recent meta-analysis of 25 DTI studies (Aoki, Abe, Nippashi & Yamasue, 2012). However, FA differences may be dependent upon age. In samples of children (Cheung et al., 2009) and early-adolescents (Cheng et al., 2010), there are also reports of increased FA in the SLF in participants with autism. There are also reports of no difference between these groups in children (Brito et al., 2009) and adults (Bakhtiari et al., 2012).

Regarding other measures, MD is commonly reported to be increased bilaterally in the SLF (Aoki et al., 2012; Shukla et al., 2010). RD of the SLF is also generally found to be increased in ASD (Ameis et al., 2010; Bloemen et al., 2010; Fletcher et al., 2010; Jeong et al., 2011; Shukla et al., 2010), but has also been found to be decreased in children (Weinstein et al., 2011). There are also reports of decreased AD in the SLF of participants with an ASD (Barnea Goraly et al., 2010).

It is clear that in autism, the nature of SLF impairments as measured by DTI is heterogeneous. Travers et al. (2011) argue several factors may account for
the highly variable findings, including the complexity of fiber geometry in this structure, methodological differences between studies (i.e. voxel versus region of interest based analyses), and participant differences (i.e. symptom profile and age range). Overall, the most common finding in the autism literature appears to be reduced FA, and increased RD and MD in the SLF.

Another WM structure relevant to autism is the cingulum bundle (CB). The CB is a pathway of WM fibres running from the anterior cingulate cortex (ACC), to the posterior cingulate, curving around the splenium and projecting to the hippocampus (Travers et al., 2011). The CB is of interest, as functional magnetic resonance imaging (fMRI) research has revealed that the ACC is one of the most commonly disturbed regions in autism (Di Martino et al., 2009). Among the many roles of the ACC including introspection at rest (De Luca, Beckmann, De Stefano, Matthews & Smith, 2006; Fox & Greicius., 2010) and executive functions (Talati & Hirsch, 2006), this region has recently been linked to MN activity using fMRI (Ramachandran & Oberman, 2006) and single cell recordings, albeit in a non-significant quantity (Mukamel et al., 2010).

In autism, the most common finding regarding WM integrity of the CB is reduced FA (Barnea-Goraly et al., 2010; Jou et al., 2010; Kumar et al., 2009; Pardini et al., 2009; Noriuchi et al., 2010), which has been linked to restricted and repetitive behaviours (Thakkar et al., 2010). One study by Cheng et al. (2010) reported increased FA in individuals with autism in the right CB, but identified a trend of age related loss in FA in participants with ASD. Thus, there is relatively strong evidence that the CB is characterized by reduced FA in ASD, particularly amongst adult populations. Nevertheless, findings from other measures have been more inconsistent. In the CB, there are reports of both
increased (Shukla et al., 2010) and decreased (Weinstein et al., 2011) RD, and
decreased AD (Nourichi et al., 2010) in participants with autism.

Thus far in this thesis, participants with autism have demonstrated
anomalies in BOLD response of the PMC and ACC (Study 1), and increased FC
between the PMC and IPL (Study 2). Given these functional anomalies in
premotor, parietal, and cingulate regions, the SLF and CB make ideal candidates
to examine structural differences in WM between these groups. Pertinent to the
hypotheses of this thesis, both the SLF (Barnea-Goraly et al., 2010) and CB
(Mukamel et al., 2010) have links to the MN network.

Consequently, the present study will utilize DTI to compare participants
with High Functioning Autism and Aspergers Syndrome (HFA/AS) to TD
participants. The primary aim of this study will be to contrast WM integrity of
the SLF and CB between the two groups. However, a secondary aim will be to
explore other WM differences between HFA/AS and TD participants distributed
across the brain.

To do this, two methods were used. Firstly, a ROI based approach will
reconstruct fibers of the SLF and CB, to contrast differences in FA and VR of
these WM structures. Fibers of the posterior limb of the internal capsule (PLIC)
will also be reconstructed, and utilized as a control condition in a similar manner
to Kumar et al. (2010). Secondly, tract-based spatial statistics (TBSS) will be
used to explore whole-brain differences in WM at the voxel level. This method
has the advantage of identifying microstructural differences in brain areas not
encompassed by the ROI based approach, and overcomes shortcomings related to
registration that are characteristic of ROI methods (Jones et al., 2005). Using
TBSS, differences in FA, MD, AD and RD between the groups will be assessed.
From these two analyses, two primary hypotheses, and a third exploratory hypothesis have been generated.

**H1:** When utilizing the ROI based approach, participants with HFA/AS will demonstrate WM impairment in the SLF and CB by comparison to TD participants.

**H2:** When utilizing the TBSS based approach, participants with HFA/AS will demonstrate WM impairment in the SLF and CB by comparison to TD participants.

**H3:** When utilizing the TBSS based approach, participants with HFA/AS will demonstrate WM impairments distributed across the brain compared to the TD group.

### 5.2 Method

#### 5.2.1 Participants

This study received ethics approval from the Deakin University Human Research Ethics Committee (DURHEC 135-2009). As the National Health and Medical Research Council covered this ethics approval, it also conformed to the Austin and Repatriation Medical Centre Ethics Committee, which covers fMRI scans at the Melbourne Brain Centre (Austin Health, Melbourne, Australia). The present study utilized the same cohort as Study (1) and (2), comparing 12 TD participants with 12 individuals who had been diagnosed with either High-Functioning Autism (HFA) or Asperger’s Syndrome (AS). All Participants were male, within a similar age range, and had been diagnosed by an experienced Clinical psychologist in the autism field using DSM-IV (APA, 2000) criteria. A
more detailed description of participants is provided in section 3.2.1, and in Table 3.1.

5.2.2 fMRI Acquisition and Pre-processing

Participants lay flat on the bed of the scanner with their head placed within the head coil. Cushions around the head coil restricted head movement. All MRI images were conducted in a 3T Siemens Tim Trio scanner (Erlangen, Germany) with a birdcage quadrature head-coil. Whole-brain blood-oxygen-level-dependent (BOLD) weighted fMRI images were acquired using a gradient-recalled echo-planar imaging (EPI) sequence (TR = 3.0 s; TE = 40 ms; flip angle = 60°; FOV = 24 x 24 cm; slice thickness = xx, 128 x 128 matrix). The DTI sequence was performed with the following parameters: TR = 8000, TE = 90, 25 diffusion encoding directions, slice thickness = 2.5 mm, percent phase field view = 100 mm, acquisition matrix = 96 0 0 96, b value = 1,000 s/mm2, with five acquisitions for each run with b = 0 s/mm2).

5.2.3 Region of Interest Tractography

The DTI data sets were transferred to a windows platform PC, where diffusion images were reconstructed, and directionally encoded FA maps calculated using DTI studio (Jiang et al. 2006). The methods employed were in accordance with previous research conducted by Spitz et al. (2013). To begin with, all participants’ diffusion images were corrected for motion and eddy current distortions. Following this, four a priori ROIs were manually chosen based upon previous research (Barnea-Goraly et al., 2010; Mukamel et al., 2010). These were the bilateral SLF, and CB. The posterior limb of the internal capsule
(PLIC) was selected as a control region, bilaterally. These tracts are depicted in Figure 5.1.

The six ROIs were manually drawn for each participant. An ellipsis was drawn around a seed point for each of the six tracts on directionally encoded FA maps. In accordance with Spitz et al. (2013), the SLF was also drawn from the coronal plane, and identified as an intense green, triangular shaped tract (Figure 5.1A). The SLF seed corresponded approximately to the middle of the PLIC in the coronal plane. The CB was drawn from the coronal plane, in the middle of the splenium or genu of the corpus callosum in the sagittal plane (Figure 5.1B). The PLIC was drawn in the axial plane, and identified as a deep blue tract (Figure 5.1C). The PLIC was used as a control condition, similarly to Kumar et al. (2010).

Tract reconstruction was performed using the fiber assignment by continuous tracking (FACT) method (Mori and van Zijl, 2002; Mori, Wakana & van Zijl, 2005). Tracts were linearly propagated based on the orientation of the largest principal axis. Line propagation was discontinued based on the standard criteria, using an anisotropy threshold of FA 0.2, and a maximum angle of change 75° between pixels. This prohibited angles larger than 41 degrees during tracking (Wakana et al., 2007). Lastly, the WM atlas provided by Mori et al. (2005) was used to assess the accuracy of tracked ROIs. From this a number of outcome measures were derived for each of the six tracts seeded. These were mean and standard deviation for FA and volume ratio (VR), number of fibres and average length of each fibre in the tract.

To assess the reliability of tract reconstruction, two assessors (TP and JM) from different research institutes independently reconstructed the six seed points
for three subjects. An intra-class correlation coefficient was then calculated to assess the degree of agreement in FA derived from fiber reconstruction by the two assessors. Independent samples t-tests were then used to contrast FA and VR values between the two groups, for each of the six seed regions.

**Figure 5.1.** In the top portion of this Figure are examples of the reconstructed fibres for the three regions which ROI tractography was undertaken. A. The superior longitudinal fasciculus, B. the cingulum bundle, and C. the posterior limb of the internal capsule. Below each ROI are the seed points that were used to reconstruct the fibres. These seed points are highlighted in yellow, and were based upon previous research (Mori et al., 2005; Spitz et al., 2013).

**5.2.4 Tract Based Spatial Statistics**

DTI data sets were analyzed with FSL 5.0 (Functional Magnetic Resonance Imaging of the Brain Software Library; Smith et al. 2004). Raw DICOM images for each participant were converted into a single, multivolume Neuroimaging Informatics Technology Initiative (NIFTI) files using MRICron (Chris Rorden, Columbia, SC, USA. www.mricro.com), enabling TBSS to be performed. Analyses were undertaken using Smith et al.’s (2006) protocol. Using the FDT diffusion module, all participants’ data was corrected for gradient coil
eddy current distortions. This was done by registering the diffusion-weighted images to a non-diffusion weighted image by affine transformation. Whole-brain mask files were created and manually edited for each participant brain using the draw and erase tools in FSL view (http://www.fmrib.ox.ac.uk/fsl/fslview/).

Whole brain voxel-wise statistical analysis of the FA, MD, RD and AD data was carried out using TBSS. The TBSS method constructs a WM “skeleton”, which is restricted only to the center of major WM tracts. Using both linear and non-linear alignment, participants’ FA images were registered into standard space using the FMRIB58 FA template. Each participant’s individual FA values were mapped onto this skeleton to permit group comparisons. TBSS has the advantage of minimizing potential misalignment problems of other voxel-based methods when analyzing diffusion data.

Each participant’s aligned FA image was projected onto the FA skeleton to correct for residual misalignments. An FA value of 0.2 was used as a threshold for the FA skeleton, to exclude tracts with high inter-individual variability, those containing a high level of partial volume, and those consisting of grey matter or CSF. This threshold has been commonly used in past research (i.e. Cheng et al., 2010; Spitz et al., 2013). This was achieved by calculating the difference between the skeletonized tracts and the WM tract centers in each individual image. The averaging procedure constrains the skeleton to exclude tracts at the outermost edges of the cortex, which effectively excludes parts of the brain where good tract correspondence cannot be achieved.

Voxelwise statistics were then undertaken using the general linear model to compare differences in FA, RD, AD and MD between the two groups. The ‘randomise’ tool was used to conduct significance testing, applying a threshold-
free cluster enhancement (Smith & Nichols, 2009) with 5,000 permutations. For FA, thresholds of $p<.005$, $p<.01$ and $p<.05$ were examined, corrected for multiple comparisons across space. This method has a high level of sensitivity to true differences while minimizing false positives, by avoiding the specification of a subjective cluster-forming threshold (Smith & Nichols, 2009). The most probable anatomic localization of each voxel cluster was determined using the FSL atlas tool (http://www.fmrib.ox.ac.uk/fsl /fslview/atlas-descriptions.html/), which incorporates several anatomic templates, including the Talairach atlas, MNI structural atlas, Julich histological atlas, Oxford thalamic connectivity atlas, Harvard-Oxford cortical and subcortical structural atlases, and the Johns Hopkins University DTI-based WM atlases.

5.3 Results

5.3.1 Region of Interest Tractography

5.3.1.1 Inter-rater reliability

Prior to conducting any between groups analysis, inter-rater reliability was assessed for three random participants using the intra-class correlation coefficient. Results of this analysis revealed near perfect agreement between the two raters for the SLF (.94), and very strong agreement for the CB (.88) and PLIC (.81). The overall intra-class correlation coefficient for these three regions was .94.
5.3.1.2 Between-Groups Analysis

The results for each of the 6 reconstructed tracts are presented in Table 5.1. Although participants in the HFA/AS group demonstrated lower FA and VR scores than the TD group for all ROIs using this approach, none were found to be significantly different. Thus, the ROI based approach did not provide evidence of gross differences in WM integrity of the SLF, CB and PLIC, between the two groups.

Table 5.1. Mean and standard deviations of fractional anisotropy (FA) and volume ratio (VR) statistics for the six seed regions. Results are shown for typically developing (TD) and high functioning autism and Aspergers Syndrome (HFA/AS) subjects. The seed regions are Superior Longitudinal Fasciculus (SLF), Cingulum Bundle (CB) and the Posterior Limb of the Internal Capsule (PLIC).

<table>
<thead>
<tr>
<th></th>
<th>TD</th>
<th>HFA/AS</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLF R</td>
<td>FA .51 ± .11</td>
<td>.50 ± .11</td>
<td>.16</td>
</tr>
<tr>
<td></td>
<td>VR .30 ± .14</td>
<td>.29 ± .13</td>
<td>.18</td>
</tr>
<tr>
<td>SLF L</td>
<td>FA .51 ± .12</td>
<td>.50 ± .12</td>
<td>.29</td>
</tr>
<tr>
<td></td>
<td>VR .31 ± .14</td>
<td>.30 ± .13</td>
<td>.37</td>
</tr>
<tr>
<td>CB R</td>
<td>FA .50 ± .13</td>
<td>.50 ± .13</td>
<td>.50</td>
</tr>
<tr>
<td></td>
<td>VR .29 ± .15</td>
<td>.28 ± .15</td>
<td>.54</td>
</tr>
<tr>
<td>CB L</td>
<td>FA .55 ± .15</td>
<td>.54 ± .14</td>
<td>.76</td>
</tr>
<tr>
<td></td>
<td>VR .34 ± .18</td>
<td>.34 ± .18</td>
<td>.65</td>
</tr>
<tr>
<td>PLIC R</td>
<td>FA .58 ± .15</td>
<td>.57 ± .15</td>
<td>.17</td>
</tr>
<tr>
<td></td>
<td>VR .39 ± .20</td>
<td>.37 ± .20</td>
<td>.13</td>
</tr>
<tr>
<td>PLIC L</td>
<td>FA .57 ± .15</td>
<td>.56 ± .15</td>
<td>.11</td>
</tr>
<tr>
<td></td>
<td>VR .38 ± .20</td>
<td>.36 ± .20</td>
<td>.09</td>
</tr>
</tbody>
</table>

5.3.2 Tract Based Spatial Statistics

5.3.2.1 Fractional Anisotropy

The TBSS analysis revealed brain-wide reductions in FA in the HFA/AS group (see Table 5.2). Using a very conservative threshold ($p<.005$), reduced FA in the HFA/AS group was only observed in the left hemisphere. These differences were situated predominantly along WM tracts in the anterior thalamic radiation nearby the inferior parietal lobule, SLF and UF nearby the insula, frontal occipital fasciculus nearby the middle frontal gyrus, and inferior
longitudinal fasciculus nearby the occipital lobe. Several reductions in FA were also observed in sub-cortical structures such as the anterior and posterior limb of internal capsule (nearby putamen), and anterior thalamic radiation (nearby the brainstem).

At $p<.01$, the HFA/AS group demonstrated reduced FA predominantly in the left hemisphere in the cingulum bundle (anterior and posterior subdivisions), SLF (inferior frontal gyrus) and UF (frontal orbital cortex), along with several subcortical structures such as the thalamus and anterior thalamic radiation bilaterally. At $p<.05$, reduced FA in the HFA/AS group was observed bilaterally, with a number of clusters in motor and sensory areas of the SLF and frontal parts of the UF. The corpus callosum also demonstrated reduced FA in HFA/AS participants bilaterally. Differences between the groups in FA at $p<.05$ are depicted in Figure 5.2. In this study, no significant increases in FA among the HFA/AS group compared to TD participants were found, even at the low threshold of $p<.05$. 
Figure 5.2. Group differences in FA (TD greater than HFA/AS) and RD (HFA/AS greater than TD), thresholded at p<.05, corrected for multiple comparisons. A large number of WM structures demonstrated increased FA in TD participants, with more pronounced differences observed in the left hemisphere. In regard to RD, HFA/AS participants demonstrated increased RD, which was lateralized to the left hemisphere.
Table 5.2. Outline of WM structures and their corresponding cortical regions that were demonstrated increased FA in TD participants compared to HFA/AS participants at p<.005, p<.01 and p<.05, correct for multiple comparisons. The far right hand columns indicates corresponding coordinates that demonstrated increased MD in individuals with HFA/AS with a * at p<.05, corrected for multiple comparisons.

<table>
<thead>
<tr>
<th>HFA/AS compared to TD</th>
<th>Cortical Region</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>p&lt;.05</th>
</tr>
</thead>
<tbody>
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<td>FA for increased FA</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
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<td>-39</td>
<td>0</td>
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<tr>
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</table>
5.3.2.2 Other Measures

Measures of MD, RD and AD were evaluated at \( p < .05 \). Overall, MD and AD did not demonstrate significantly different values in either group. However, RD was increased in participants with HFA/AS, as can be seen in Table 5.2. There were a number of co-ordinates that possessed greater RD, which overlapped with co-ordinates demonstrating reduced FA in the HFA/AS group. These include the SLF nearby the insula, IPL and precentral gyrus, and subcortical structures along the anterior thalamic radiation (thalamus, brainstem and caudate) in the left hemisphere. Significant differences in RD between the groups were not observed in the right hemisphere (Figure 1). The TD group did not demonstrate increased RD in any voxels at \( p < .05 \), corrected for multiple comparisons.

5.4 Discussion

In this study, there were two aims. Firstly, to contrast WM tracts that may be associated with the MN system between TD and HFA/AS participants (the SLF and CB). Secondly, to explore any other differences in WM integrity between the two groups, across the brain. The first aim was assessed with ROI (H1) and TBSS (H2) based methods, whilst the latter aim was assessed only with TBSS (H3).

The first hypothesis that the ROI based approach would reveal impaired WM integrity in the HFA/AS group in the SLF and CB was not supported. Participants with HFA/AS demonstrated no significant differences in FA or VR of these two regions compared to the TD group. However, it is notable that non-
significant reductions in FA were observed bilaterally for all ROIs in the HFA/AS group (see Table 5.1). Thus, the trend of the findings was consistent with previous ROI based investigations implementing similar parameters with DTI studio (Sundaram et al., 2008; Thomas et al., 2011). To date, only Kumar et al. (2010) has looked specifically at the SLF and CB using DTI studio, and reported significant, but small reductions in FA of the left SLF and right CB in the autism group. It is notable this study scanned 32 participants with autism compared to 12 in the present study, which may suggest a larger sample size would have yielded significant results. Further, a limitation of ROI based analyses is they provide FA values of gross WM structures, and are therefore less sensitive to subtle differences in WM that may be present in sub-divisions of these structures.

Consequently, TBSS was also undertaken to examine differences in WM integrity voxel by voxel, across the brain. Using this method, the second hypothesis that participants with HFA/AS would demonstrate WM impairments in the SLF and CB was supported. Reduced FA and increased RD were identified in the SLF nearby several cortical regions, including the insula, inferior frontal gyrus, supramarginal gyrus, middle temporal gyrus and pre and post central gyrus in the left hemisphere, and the supramarginal gyrus in the right hemisphere. This supports a number of previous investigations to identify reduced FA (Barnea Goraly et al., 2010; Bloemen et al., 2010; Jou et al., 2010; Lee et al., 2007, Shukla et al., 2010) and increased RD (Ameis et al., 2010; Bloemen et al., 2010; Fletcher et al., 2010; Jeong et al., 2011; Shukla et al., 2010) in the SLF.

Of particular interest to this thesis are the observed reductions in FA in the SLF III bilaterally, corresponding to the supramarginal gyrus. Barnea Goraly et al. (2010) also reported reduced FA in SLF III, and suggest this may be linked
to abnormal MN function. The IPL, overlapping with the supramarginal gyrus is well established to form part of the MN network (Rizzolatti, Fadiga, Gallese & Fogassi, 1996; Rizzolatti & Luppino, 2001). In fMRI research, it has also demonstrated increased activation during imitation paradigms (Dapretto et al., 2006; Wiliams et al., 2006).

Using TBSS, reduced FA was also observed in the CB, with both the anterior and posterior section demonstrating differences in the left hemisphere, and just the posterior section in the right hemisphere. Several prior investigations have reported reduced FA in the CB (Barnea-Goraly et al., 2010; Jou et al., 2010; Kumar et al., 2010; Pardini et al., 2009; Nourichi et al., 2010; Thakkar et al., 2010). Increased RD was observed in the left posterior part of the CB, supporting one past study (Shukla et al., 2010). Although the CB has not been directly linked to MN function, these findings are of interest to the present study, given a recent single cell study identified that 21% of measured cells responded to action observation in the dorsal ACC, and 17% in the rostral ACC (Mukamel et al., 2010). It must be acknowledged that these quantities did not reach significance however.

The third hypothesis that the HFA/AS group would demonstrate widespread WM impairment across the brain was also supported. Among TD participants, FA was increased in a distributed network of WM in cortical (the uncinate fasciculus, inferior longitudinal fasciculus, frontal-occipital fasciculus, optic radiation, cerebral peduncle) and sub-cortical (anterior thalamic radiation) regions. Brainwide reductions in FA across the brain are commonly reported in ASD (Alexander et al., 2007; Barnea-Goraly et al., 2004; Keller et al., 2007; Lee et al., 2007; Thakkar et al., 2008), and have been argued to reflect abnormal
neurodevelopment of WM skeletons in childhood and adolescence (Cheng et al., 2010). Abnormal neurodevelopment likely stems from an abundance of problems including inappropriate synaptogenesis and synapse elimination (Cheng et al., 2010), disturbed neural migration (Blaylock, 2008; Schmitz & Rezaie, 2007), disorganized minicolumns (Cassanova et al., 2006) and anomalies at white-gray mater boundaries (Avino & Hustler, 2010).

Strikingly, when a conservative significance threshold of p<.005 was implemented, increased FA in the TD group was confined to the left hemisphere. Although theories that autism is characterized by left hemisphere impairment date back nearly 30 years (i.e. Hier, LeMay & Rosenberger, 1979), they have received renewed interest with advances in neuroimaging. Research utilizing DTI (Fletcher et al., 2010; Lange et al., 2010; Nagae et al., 2012), diffusion spectrum imaging (Lo et al., 2011), and volumetric analyses (Rojas, Bawn, Benkers, Reite & Rogers, 2002) all suggest participants with autism may possess more pronounced deficits in the left hemisphere. One recent study has even suggested that volumetric and morphological features of the left hemisphere specifically, are most reliably able to diagnostically distinguish TD from autistic participants (Ecker et al., 2010).

Although the functional significance of left hemisphere impairments in autism requires further research, it may be linked to impairments in language. Past research on TD samples has demonstrated left laterality in FA values (Catani et al., 2007; Vernooij et al., 2007), which has been argued to underline hemispheric specialization in language (Tavers et al., 2012). There is evidence this lateralization is reduced in autism, specifically in the left SLF and uncinate fasciculus (Fletcher et al., 2010; Lange et al., 2010; Lo et al., 2011). Moreover,
Nagae et al. (2012) report a significant, but small negative correlation between reduced MD of the left SLF, and increases in language skills ($R^2 = 0.2$). Nevertheless, similar findings have been made in schizophrenia (Sommer, Ramsey, Kahn, Aleman & Bouma, 2001), which may suggest left hemisphere deficits are common to clinical populations, or represent a generalized deficit in sensory processing.

In the present study, a clue as to what specific neurodevelopmental anomalies have occurred in the HFA/AS group may come from the finding of deficits in RD, in the absence of deficits in AD and MD. Research utilizing animal models provides evidence that increases in RD are more sensitive to demyelination, where as AD is sensitive to axonal damage (Budde et al., 2007; Song et al., 2005). This raises the possibility that deficits in myelin may underpin the findings for the HFA/AS group in this study.

However, myelination problems have not typically been associated with ASD. In one of the few studies to directly assess myelination, Kemper and Bauman (1998) report no differences in myelination between ASD and TD post-mortem brains. This finding may not be conclusive however, as Sundaram et al. (2008) points out that the myelin staining technique implemented in this study (Loyesz method) is only sensitive to myelin deficiencies. Subtle, quantitative disturbance to myelin such as increased thickness could not be accounted for. Importantly, increased RD can be indicative of myelin disturbance that includes increased thickness, in addition to smaller axonal diameter, and reduced extracellular space (Gao et al., 2009).

Two previous DTI studies report increased RD (Ameis et al., 2010; Alexander et al., 2007), whilst another study utilizing a much younger sample
(aged >13) reported reduced RD (Barnea-Goraly et al., 2010). Further, Herbert et al. (2004) identified increased WM volume in participants with autism in regions of the brain known to myelinate late in development (i.e. prefrontal cortex). These preliminary findings raise the possibility that myelin disturbance may be localized to specific brain regions, in later periods of brain development in ASD. In turn, these impairments may impact the speed and synchronization of signal transmission along WM tracts (Ameis et al., 2010).

In sum, although the ROI based approach did not reveal differences between the groups, TBSS revealed reduced FA, and increased RD in individuals with HFA/AS compared to TD participants. These differences were observed in hypothesized regions (the SLF and CB) which may have relevance to the MN system, but also in widespread regions across the brain (anterior thalamic radiation, uncinate fasciculus, inferior longitudinal fasciculus, frontal occipital fasciculus). These impairments were more pronounced in the left hemisphere, and characterized by increased RD which may indicate myelination disturbances. It is argued that an abnormal period of neurodevelopment, which specifically impacts myelin structure, may be responsible for the present results.
Chapter 6: General Discussion
6.1 Overall Summary

The present thesis assessed the mirror neuron (MN) hypothesis of autism, where a single sample of typically developing (TD) and high functioning autism or aspergers syndrome (HFA/AS) participants were contrasted with three MRI techniques; task-dependent activation, functional connectivity (FC) and structural connectivity. The sample participant cohort was used across each of the three experiments. The goal of this thesis was to thoroughly characterize cortical differences in both function and structure of the MN system between these groups.

There were two important findings to arise from this dissertation. Firstly, in participants with HFA/AS, there is evidence of both functional and structural anomalies in a network of cortical regions believed to possess MNs. That is, abnormalities were present during a task designed to engage these regions (observation of hand actions), and also during experiments designed to assess putative connections between regions in this network.

Secondly, despite these observed anomalies in the MN system of participants with HFA/AS, this does not appear to be a parsimonious explanation of the brain-wide distribution of differences observed between the groups. MN abnormalities in autism appear to be one part of large-scale differences in functional and structural networks, encompassing both cortical and sub-cortical regions. Although speculative based upon the data of this thesis, there are several theories in the literature that may provide a more inclusive explanation of the overall findings.
On this basis, the goal of this discussion will be to (a) explain the relevance of the present findings to the MN hypothesis of autism and contrast with past literature, and (b) speculate on broader explanations for the brain-wide distribution of differences observed in participants with HFA/AS.

### 6.2 Relevance to Mirror Neuron Hypothesis of Autism

#### 6.2.1 Study 1

Study 1 contrasted task-dependent change in blood oxygen level dependent (BOLD) between TD and HFA/AS participants. Utilizing a block design, observation of active hand actions was contrasted with observation of a still hand. The key finding related to the MN hypothesis of autism was that HFA/AS participants demonstrated increased activity in right, dorsal premotor cortex (PMC).

Although MNs were first discovered in the ventral PMC, corresponding to area F5 in Macaque Monkeys (Gallese, Fadiga, Fogassi and Rizzolatti, 1996), the dorsal PMC is considered part of the extended MN system (Caspers et al., 2010). Previous EEG research provides evidence for a general role of the PMC in MN tasks (Muthukumaraswamy & Johnson, 2004; Arnstein, Cui, Keysers, Mauritz & Gazzola, 2011), whilst micro stimulation research confirms it is somatotopically linked to the hand (Raos, Franchi, Gallese & Fogassi, 2003). Thus, given prior research, and the design of this study, it is likely the regional increase in BOLD of the right PMC overlaps with MN circuits.

Only one previous study investigating MN function has reported increased dorsal PMC in autism, but this was during imitation (Williams et al.,
2006). These authors speculate that participants with ASD have a stronger reliance upon visuomotor learning than TD participants. There is some direct evidence that participants with an ASD demonstrated increased PMC activity when engaged in a visuomotor task (Muller, Cauich, Rubio, Mizuno & Courchesne, 2004).

Most of the previous research for PMC anomalies during MN tasks in autism comes from EEG. Several authors studying hand actions (Martineau, Cochin, Magne & Barthelemy, 2008; Oberman, et al., 2005; Oberman, Ramachandran & Pineda, 2008) report that mu suppression (considered an indirect indicator of MN activity) is reduced or absent in participants with an ASD. Reductions in mu are believed to be due to dysfunctional inputs from premotor and parietal MNs (Muthukumaraswamy & Johnson, 2004; Arnstein et al., 2011). Thus, there is some converging evidence of premotor abnormalities in autism, which may be linked to problems with the MN system.

From this thesis, and previous fMRI and EEG research, it is not possible to determine the precise neural mechanisms that are dysfunctional in the PMC. However, there is preliminary evidence from transcranial magnetic stimulation research that those with autism may possess faulty inhibitory mechanisms in motor regions (Enticott, Rinehart, Tonge, Bradshaw & Fitzgerald, 2012). Specifically, these authors found that participants with HFA demonstrated reduced GABA_A, a finding that has also been made in post-mortem autistic brains (Collins et al., 2006; Fatemi et al., 2009).

Supporting these findings, a theory has been postulated that participants with autism possess an imbalance in excitatory and inhibitory mechanisms in the frontal lobe (Rubenstein & Merzenich, 2003). Thus, it could be speculated that
the increase in BOLD signal observed in this study, and reductions in \textit{mu}
suppression observed in past EEG research, are attributable to over-activation of
inhibitory circuit in premotor areas.

It must be acknowledged that several past studies investigating the MN
network in autism have reported no difference in activation of MN regions
compared to TD participants (Dinstein et al., 2010; Marsh & Hamilton, 2011).
However, these two studies utilized different control conditions to the present
experiment. Where the present study utilized a still hand, Dinstein et al. (2010)
utilized a blank screen, whilst Marsh and Hamilton (2011) utilized non-biological
movement. These differences are likely to contribute to the discrepant findings.

Further, the experiment of this thesis only utilized an observation
condition, where previous research recommends that BOLD signal during
observation \textit{and} execution should be explicitly linked to quantify MN activity
(Keysers, Thioux & Gazzola, 2013). Thus, although the observation condition
was designed to elicit a MN response, more discerning measures such as pattern
classification may have been more sensitive to explicitly measuring the MN
network (Etzel, Gazzola & Keysers, 2008).

Increased activation in participants with HFA/AS was also observed in
the anterior cingulate cortex (ACC). Drawing upon past research to have mapped
the ACC with fMRI (Marguiles et al., 2007), the peak coordinate in this study to
demonstrate increased activity in participants with HFA/AS was in a subgenual
portion, corresponding approximately to the rostral ACC.

Importantly, there is growing evidence that the ACC may possess MNs.
In a meta-analysis of 125 fMRI MN studies, the right ACC was one of 14
significant clusters to be attributed to MN functioning in humans (Molenberghs
et al., 2011). Furthermore, the only single cell study to date to have measured MNs in humans, reported that 17% of neurons measured in the rostral ACC discharged in response to action observation (a non-significant finding however). Although based on limited evidence, it is possible the increased activation in this region among participants with HFA/AS may be attributable to MN function. The ACC, along with the insula is believed to form a separate MN network linked to emotional processing (Gallese, Keysers & Rizzolatti, 2004; Rizzolatti & Fabbri-Destro, 2010; Rizzolatti, Fabbri-Destro & Cattaneo, 2009).

Nevertheless, the ACC is known to have multiple roles including response inhibition (Agam, Joseph, Barton & Manoach, 2010), learning (Bush, Luu & Posner, 2000) and error-detection (Bush et al., 2002), making it difficult to infer exactly what this activation might reflect. Moreover, in a review of non-social tasks, Di Martino et al. (2009) report that the rostral ACC and supplementary motor area are the most common brain regions to activate abnormally in fMRI research in autism. Thus, differences in the ACC among autism participants are not limited to MN investigations. However, given differences in the ACC were observed during a MN task, it is plausible that it reflects MN activity.

6.2.2 Study 2

Based upon the findings of Study 1, Study 2 utilized two MN regions as seed points to assess FC across the whole brain during a resting-state scan. These were the right dorsal PMC, and right ACC, and utilized the precise coordinates which demonstrated increased activity in the HFA/AS group in Study 1. When seeding the right PMC, participants with HFA/AS demonstrated increased FC
with the left inferior parietal lobule (IPL). Between these regions, a positive correlation in FC was observed for both groups, with the magnitude of the correlation being significantly larger in the HFA/AS group.

The relevance of this finding to the MN hypothesis of autism is somewhat speculative. There are a large number of parallel neural systems connecting frontal and parietal cortices, which have a variety of roles including voluntary movement, and sensori-motor integration (Iacoboni & Dapretto, 2006). There is evidence that the MN system constitutes one of these networks, linking the inferior frontal gyrus, PMC and inferior parietal lobule (IPL) (Buccino, Binofski & Riggio, 2004; Rizzolatti & Craighero, 2004). However, FC is not sensitive enough to distinguish the different fronto-parietal networks from one another. Nevertheless, given the seed point utilized was selected due to its activation in response to MN stimuli in Study 1, it can be inferred that the change in BOLD between the groups is constituted in part by the fronto-parietal MN circuit.

At present, very few studies have examined FC with the specific intent of looking at MN regions in participants with autism. Moreover, meaningful comparisons between studies are hindered by important methodological differences. For example, a study by Rudie et al. (2011) reported reduced FC in a front-parietal network among participants with autism. Although this is the opposite finding to this thesis, Rudie et al. (2011) utilized a different seed point (the right pars opercularis), and investigated FC that was specifically related to a task (as compared to resting-state FC in this thesis). Similarly, Villa-Lobos et al. (2005) also reports reduced FC in areas linked to the MN system, but their study differed to the present study methodologically (utilization of a different seed point and use of task-regression).
A recent meta-analysis of 32 FC studies in autism by Muller et al. (2011) has confirmed that different design decisions related to filters, seed selection and task all contribute to different patterns of FC in participants with autism. For example, task-based FC studies more commonly report reduced FC in autism, whilst resting-state scans tend to find a mix of reduced and increased FC. Thus, it is difficult for the present findings to be compared to past research to investigate FC in MN regions, given these crucial methodological differences.

Study 2 also revealed that TD participants demonstrated increased FC between the right PMC and a small cluster situated nearby the left insula. This difference was characterized by a tiny, positive correlation in FC between these regions in the TD group, and a small anticorrelation in FC for the HFA/AS group. The insula is considered an important bridge between the fronto-parietal MN system, and the limbic system (Iacoboni, 2007; Iacoboni & Dapretto, 2006). However, the significance of anticorrelations in FC studies is a contested point; with some authors arguing it reflects intrinsic neural processes (Fox et al., 2005), whilst others suggest it may reflect a methodological artifact (Murphy, Birn, Handwerker, Jones & Bandettini, 2009). Thus, this finding must be interpreted cautiously.

Nevertheless, a comparable finding comes from Ebisch et al. (2010). In this study they seeded the anterior and posterior insula, and reported overall reduced FC in autism with a large number of frontal regions. The findings of Ebisch et al. (2010), along with the findings of this thesis provide some preliminary evidence of reduced FC between the insula and frontal regions in autism. Although it remains to be seen if this finding can be replicated, it is notable that in a meta-analysis of 24 studies, the insula was found to be a
consistent locus of hypoactivity (Di Martino et al., 2009), which has led to a
theory that under-connectivity to the insula has an important role in dysfunctional
emotional processing in autism (Uddin & Menon, 2009). Thus, when considering
both task-dependent and FC research, there is evidence that participants with
autism possess a reduced cortical response in the insula, which may be linked to
empathy and affective processing.

When using the ACC as a seed, no differences were observed between the
two groups in whole-brain FC. However, previous research investigating FC in
the ACC has identified both increased and decreased FC. Using the superior
frontal gyrus as a seed, Shih et al. (2010) report increased FC with the ACC in
participants with autism, whilst a seed based analysis during a resting state scan
by Cherkassky, Kana, Keller and Just (2005) reported reduced FC in autism
between the anterior and posterior cingulate cortex.

Thus, there do not appear to be consistent findings in regard to FC from
the ACC in participants with autism. As mentioned, these mixed effects between
studies are most likely influenced by differences in methodology and design
(Muller et al., 2011). A further contributor to the null finding in this thesis is the
use of a band-pass filter. This limits the band of the signal measured to avoid
methodological artifacts and cognitively induced fluctuations, but can induce
noise that leads to false negative results (Damoiseaux et al., 2009). Although this
can be avoided by using independent component analysis, this can reverse the
problem, and lead to false positives related to artifact noise.
6.2.3 Study (3)

Using DTI, Study 3 assessed the structural properties of two WM fasciculi linked to the MN system: the superior longitudinal fasciculus (SLF) and cingulum bundle (CB). These fasciculi were assessed with two techniques. The first technique was a ROI based analysis that involved fibre reconstruction, and found no gross differences in FA between the two groups in these WM fasciculi. The second technique utilized tract based spatial statistics (TBSS) to examine WM pathways voxel by voxel across the entire brain, and identified reduced FA, and increased radial diffusivity (RD) in the SLF and CB of participants with HFA/AS.

Similarly to the findings of Study 2, these two WM structures are highly complex, and subsume multiple neural systems. Although the over arching function of the SLF is multisensory association between the frontal and parietal lobes (Makris et al., 2005), it has been speculated that the SLF III may partly be comprised of MNs originating from the IPL and terminating in the ventral PMC (Barnea Goraly et al., 2004). In Study 3, the TBSS analysis revealed reduced FA bilaterally, in a region roughly corresponding to the SLF III, nearby the supramarginal gyrus. In the left hemisphere, increased RD was observed in the same coordinate. Although reduced FA was also observed along other parts of the SLF (pre and post central gyrus), it was not observed in the PMC.

Thus, in participants with HFA/AS, anomalies in SLF with relevance to MN regions were only identified in the IPL. In fMRI research, increased parietal activity in participants with autism has been observed during imitation paradigms (Dapretto et al., 2006; Williams et al., 2006). Moreover, previous DTI research into the SLF supports the finding of this thesis of reduced FA in participants with
autism (Barnea Goraly et al., 2010; Bloemen et al., 2010; Jou et al., 2010; Lee et al., 2007, Shukla et al., 2010). Taking these findings together, anomalies in the parietal node of the MN system may be linked to impaired imitation in participants with autism. Further, they may also be predominantly characterized by impaired WM integrity, as no functional anomalies were observed in Study 1 or 2. Supporting this view, Hamilton (2013) points out that most functional studies of MNs role in autism do not find parietal differences (Hamilton, 2013).

Reduced FA and increased RD were identified in the CB of participants with HFA/AS. Cortically, this corresponded roughly to the ACC, which as previously covered has been linked to the MN system (Mukamel et al., 2010). This is consistent with most research into FA of the CB (Barnea-Goraly et al., 2010; Jou et al., 2010; Kumar et al., 2010; Pardini et al., 2009; Noriuchi et al., 2010; Thakkar et al., 2010). Increased RD was observed in the left posterior part of the CB, supporting one past study (Shukla et al., 2010). Thus, this thesis provides evidence of both functional and structural anomalies in the CB of participants with HFA/AS, which could potentially be linked to the MN system.

6.2.4 Overall Implications

This thesis has provided evidence of diverse abnormalities in a network overlapping with MN regions in participants with HFA/AS. Task specific anomalies were situated in frontal nodes (right PMC and ACC), and were characterized by increased BOLD response compared to TD participants. FC was increased in HFA/AS participants between the right PMC and left IPL. Finally, impaired WM integrity was observed in the parietal node of the MN system (the SLF III, corresponding to the supramarginal gyrus) and the CB.
Given the structural deficits identified in the parietal node of the MN system (Study 3) occurred in the absence of functional differences in Study 1, it could be theorized that this is the catalyst for individuals with an ASD to undergo atypical sensorimotor learning. Motor areas receive robust sensory inputs from the IPL (Caspers et al., 2013; Iacoboni & Dapretto, 2006), so any difference in WM integrity in this region could result in downstream functional impairments to frontal nodes of the MN system. Furthermore, the responsiveness of the MN system is believed to be learnt rather innate, where simultaneous firing of premotor and parietal neurons strengthens visuomotor connections (Ferrari et al., 2013). Thus, in ASD, abnormal connectivity between these regions could potentially give rise to atypical sensorimotor experiences across the lifespan (Catmur, 2013).

Research on TD participants has revealed that the left IPL in particular may have an important role in action observation. Using EEG to examine brain microstates, Ortigue, Sinigaglia, Rizzolatti and Grafton (2010) reveal a distinct pattern of temporal activation in the MN network. They report that when observing a motor action, bilateral activation of occipital, temporal and parietal brain regions occurs between 0-120mm (microstate 1), followed by activity in the left IPL between 122-200mm (microstate 2) that coincides with almost complete disappearance of right hemisphere activation (Ortigue, Sinigaglia, Rizzolatti & Grafton, 2010). Thus an important area for future research is the temporal profile of IPL activity in autism, particularly between 122-200mm following observation of an action.

Although the findings of this thesis were complimentary to past research to have utilized similar methodologies, some caution is required applying these
results to MN theory. Notwithstanding the varied design decisions in MN research, there is not yet consistent evidence of specific, replicated differences in this network among autism participants. Across all neuroimaging techniques (task-dependent designs, FC, DTI) and neurophysiological measures (EEG), the cortical response of participants with HFA/AS has been demonstrated to be greater than, less than, or equivalent to TD participants.

Moreover, the *a priori* specified brain regions investigated in this thesis encompass relatively large cortical regions, where the proportion of cells that actually demonstrate mirror properties cannot be ascertained from these methods. Single cell studies provide evidence that the number of neurons with mirror properties in humans ranges between 5 and 33% in a given cortical region (Mukamel et al., 2010), which is fairly similar to estimates from research on Macaque Monkeys (Gallese, Fadiga, Fogassi & Rizzolatti, 1996). Two implications of this are that it is difficult to specify precise, anatomically localized MN regions (Hamilton, 2013), and that at best, differences in task-dependent BOLD, FC and FA can only be attributed to a network partly comprised of MNs.

A further caveat is that the cortical differences observed between the groups are open to other interpretations. All cortical networks and structures examined in this thesis are comprised of many parallel systems with multiple functions. For example, the SLF III is also believed to play a role in language (Bernal et al., 2010), whilst the ACC is known to have a role in executive functions (Bush et al., 2002). Both language and executive function deficits have been linked to autism, and as such these interpretations cannot be discounted.
Thus, although experiments were designed in such a way as to maximize the likelihood of measuring MN regions, alternate interpretations remain possible.

Finally, for all three studies, differences in MRI signal were also observed in regions not believed to constitute MNs. Even if it is assumed that the previously discussed differences reflect the MN network, this is not a full characterization of brain differences observed in this thesis. For example, in the task-dependent MN study, differences between the groups were observed in the cuneus, calcarine and middle temporal gyrus. Thus, it doesn’t appear that the profile of the autistic brain can be parsimoniously explained by abnormal task response or connectivity in the MN system exclusively. Given this point, the next section will discuss a number of theories that provide a more inclusive explanation of the cortical differences observed between the two groups.

### 6.3 Alternative Explanations

It is clear from this thesis and previous research, that participants with autism demonstrate widespread brain anomalies that encompass multiple brain systems. This general characterization of brain function in autism appears to apply both to highly focused studies of task performance, and broader studies of anatomical connections. Thus, there is a need for more parsimonious theories, which are capable of explaining this broad profile of brain anomalies.

One suggestion is that perhaps autism is characterized by ‘cortical inefficiency’ (Dichter, Felder & Bodfish, 2009). This subsumes a myriad of problems which may stem from less selective neuroplasticity over the course of neurodevelopment (Lewis et al., 2009). In turn, this may result in redundantly
activated cortical regions (Buchsbaum et al., 2007), imbalances in excitation and inhibition (Rubenstein & Merzenich, 2003), a critical dependence on magnitude of activation during tasks (Karlsgodt et al., 2007), disturbances in corticospinal excitability (Oberman et al., 2012) and numerous other brain-wide anomalies.

It is possible that cortical inefficiency may contribute to the brain-wide differences in cortical function and structure observed in participants with HFA/AS in this thesis. It could also potentially explain the seemingly paradoxical finding of increased FC between the premotor cortex and IPL, but reduced structural connectivity in the IPL of participants with HFA/AS. For example, increased FC in the HFA/AS group may be a compensatory neural response that stems from reduced WM integrity.

However, a caveat to this theory is that cortical inefficiency has been applied to many other disorders such as major depressive disorder, bipolar disorder, and schizophrenia (Dichter et al., 2009). Thus, it does not provide an adequate explanation for unique aspects of autism’s symptom profile. It may be the case that cortical inefficiency represents a fairly general form of brain dysfunction, which is common to heterogeneous disorders (Monoach, 2003).

Thus, in autism research, there is a need to identify whether there are abnormal neural processes that modulate differences in multiple networks such as the MN system, which are also relatively unique to the disorder. One recent suggestion is that participants with autism may possess poor cortical response reliability. In other words, neural responsiveness to external stimuli may be inherently more variable in this population. Using fMRI, Dinstein et al. (2012) had participants observe a basic visual, auditory and somatosensory stimulus over repeated trials, and found those with autism possessed larger variation in activity
of visual, auditory and somatosensory cortices. Although only a preliminary finding, this may be a promising avenue of research that can explain the lack of replication in functional neuroimaging research of autism participants.

Although these authors also report no difference between the groups when examining task-free brain activity, it remains an open question whether a similar response could be observed in a study of FC across the brain. If response reliability were to be assessed in a FC study, it would raise questions regarding how flexibly the autistic brain can adapt to external change. In TD samples, there is evidence that functional networks can reconfigure themselves on the timescale of a few 100 milliseconds in response to changing environmental demands (Honey, Cotter, Breakspear & Sporns, 2007; Honey et al., 2008). This has been attributed to ‘catalyst’ nodes across the brain, which reconfigure functional networks (during task switching for example; McIntosh, 2004). Thus, an area of future research might be to assess not just response reliability, but response flexibility in participants with autism. Poor response flexibility may underlie a general deficit in shifting attention, which could contribute to impaired development of the MN system.

DTI research has demonstrated that TD participants possess left laterality in FA values (Catani et al., 2007; Vernooij et al., 2007), which has been argued to underline hemispheric specialization in tasks such as language (Travers et al., 2012). As outlined in Study 3, this thesis demonstrated that impairments in WM were more pronounced in the left hemisphere. A number of recent studies have also identified this lateralization to be reduced in autism, specifically in the left SLF and uncinate fasciculus (Fletcher et al., 2010; Langen et al., 2010; Lo et al., 2011). Moreover, a recent study by Ecker et al. (2010) reported that volumetric
and morphological features of the left hemisphere were able to discriminate participants with autism from TD participants.

Thus, the data in this thesis supports theories that participants with autism demonstrate more pronounced deficits in the left hemisphere (i.e. Hier, LeMay & Rosenberger, 1979). Although the reasons why it is more vulnerable in autism remain unclear, it may be due to the left hemisphere being under tighter genetic control than the right hemisphere (Thompson et al., 2001), which is important to a highly heritable disorder like autism. For the most part, these WM anomalies were attributable to increased RD. This is believed to be an indirect indicator of demyelination (Budde et al., 2007; Song et al., 2005), including increased myelin thickness, smaller axonal diameter or extracellular space (Gao et al., 2009).

At present, there is a paucity of research looking at myelination anomalies in ASD. Although one previous investigation of post-mortem brains with autism report no difference in myelination compared to TD participants (Bauman & Kemper, 1998), Sundaram et al. (2008) point out that the myelin staining technique in this study (Loyesz method) is only sensitive to myelin deficiencies. Subtle, quantitative disturbance to myelin such as increased thickness could not be accounted for. Interestingly, Herbert et al. (2004) reported increased WM volume in participants with autism in regions of the brain known to myelinate late in development (i.e. prefrontal cortex), raising the possibility that myelin disturbance may be localised to specific brain regions.
A potentially important limitation of this thesis is that IQ measures were not taken. When conducting neuroimaging research into autism, IQ measures are commonly assessed, then covaried out. The rationale for this is that it determines if cortical differences between TD and autism groups are independent of intelligence differences. Thus, the influence of intelligence upon the results was not systematically controlled for in this thesis. Nevertheless, all participants with autism had a confirmed diagnosis of high functioning autism, which helped limit the range in which IQ could vary between the groups (i.e. IQ > 70). Moreover, the trend of findings in this thesis is consistent with past research to have controlled for IQ and used similar methodologies (i.e. Martineau et al., 2010 in Study 1, Monk et al., 2009 in Study 2, Barnea-Goraly et al., 2010 in Study 3). There are also authors who argue that lower IQ is part of the autistic phenotype, where strictly matching IQ can obscure differences linked to the condition itself (Courchesne, Townsend & Saitoh, 1994). However, it must be acknowledged that the results of this thesis may have differed if IQ was systematically controlled for.

A related problem is that no formal measures of autistic symptoms were assessed. These measures are useful to link anomalous brain activation to particular symptoms, such as social deficits or repetitive interests. Given that MNs are believed to have a functional role in a number of autism deficits such as empathy and imitation (Perkins, Stokes, McGillivray & Bittar, 2010), they would have value-added to the functional significance of the findings of this thesis. Nevertheless, with a relative small sample size for each group (N=12), it is
debatably whether there would have been adequate power to perform such an analysis.

Whenever undertaking neuroimaging research, there is always a risk of false positive findings. In all three studies of this thesis, analyses were conducted across the whole brain. This means a large number of voxels were tested, and by extension, a large number of statistical tests. However, all three studies utilized voxel, and-or cluster-wise control of family wise error to minimize this problem, in line with recommendations for reporting fMRI research by Poldrack et al. (2008). It has been argued that family wise error can be too conservative (Logan & Rowe, 2004). This potential problem was partly controlled by the use of small volume corrections, to increase sensitivity to hypothesized regions.

Finally, as briefly alluded to, the sample size in this thesis (N=12 in each group) was comparatively small to most neuroimaging studies of ASD. Although several precautions were taken to avoid false positive findings (error correction, long scan times and combining of conditions), statistical power is still much less than research utilizing cohorts of 50 or more in each group. This issue is shared with many other studies reviewed in this thesis, and is almost certain to contribute to the pervasive differences in findings across all neuroscientific techniques. Button et al. (2013) point out this problem pervades much of cognitive neuroscience, and recommends that data sharing will help to improve reproducibility of results.
In conclusion, this thesis examined the MN hypothesis of autism in a single sample of participants across three neuroimaging techniques; task-dependent activation, FC and DTI. This allowed for the MN network to be systematically assessed with a multifaceted approach, exploring its response to observed stimuli, the temporal relationships between cortical regions in the network, and properties of WM tracts linking the network. To the author’s knowledge, this is the first body of work to use each of these three techniques on a single sample of participants with autism.

Among participants with HFA/AS, a diverse set of anomalies was observed across the MN network, predominantly in PMC, ACC and IPL. However, these findings appear to form part of a more complex pattern of atypical brain response in autism. Overall, participants with HFA/AS demonstrated increased BOLD response in frontal regions when observing hand actions, and increased FC between frontal and parietal regions. WM integrity was impaired across the whole-brain, but more pronounced in the left hemisphere.

These brain wide differences in participants with autism may point toward a number of generalized deficits in neural processing that trace back to atypical neurodevelopment. It is speculated they may be underpinned by generalized deficits such as cortical inefficiency and impaired evoked response reliability. The results of this thesis suggest the presence of structural deficits in WM of participants with autism were more pronounced with the left hemisphere, which is likely, attributable to genetic factors.
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Appendices

Appendix A: Autism Quotient

The Adult Autism Spectrum Quotient (AQ)

Ages 16+

SPECIMEN, FOR RESEARCH USE ONLY.

For full details, please see:

The Autism Spectrum Quotient (AQ): Evidence from Asperger Syndrome/High Functioning Autism, Males and Females, Scientists and Mathematicians
Journal of Autism and Developmental Disorders 31:5-17

Name:...........................................     Sex:...........................................

Date of birth:...............................     Today’s Date:.................................

How to fill out the questionnaire

Below are a list of statements. Please read each statement very carefully and rate how strongly you agree or disagree with it by circling your answer.

DO NOT MISS ANY STATEMENT OUT.

Examples

<table>
<thead>
<tr>
<th>Number</th>
<th>Statement</th>
<th>Definitely Agree</th>
<th>Slightly Agree</th>
<th>Slightly Disagree</th>
<th>Definitely Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>I am willing to take risks.</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>E2</td>
<td>I like playing board games.</td>
<td></td>
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<tr>
<td>E3</td>
<td>I find learning to play musical instruments easy.</td>
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<tr>
<td>E4</td>
<td>I am fascinated by other cultures.</td>
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<tr>
<td>1.</td>
<td>I prefer to do things with others rather than on my own.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>2.</td>
<td>I prefer to do things the same way over and over again.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>3.</td>
<td>If I try to imagine something, I find it very easy to create a picture in my mind.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
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<tr>
<td>4.</td>
<td>I frequently get so strongly absorbed in one thing that I lose sight of other things.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>5.</td>
<td>I often notice small sounds when others do not.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>6.</td>
<td>I usually notice car number plates or similar strings of information.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>7.</td>
<td>Other people frequently tell me that what I’ve said is impolite, even though I think it is polite.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>8.</td>
<td>When I’m reading a story, I can easily imagine what the characters might look like.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>9.</td>
<td>I am fascinated by dates.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>10.</td>
<td>In a social group, I can easily keep track of several different people’s conversations.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>11.</td>
<td>I find social situations easy.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>12.</td>
<td>I tend to notice details that others do not.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>13.</td>
<td>I would rather go to a library than a party.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>14.</td>
<td>I find making up stories easy.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
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<tr>
<td>15.</td>
<td>I find myself drawn more strongly to people than to things.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
</tr>
<tr>
<td>16.</td>
<td>I tend to have very strong interests which I get upset about if I can’t pursue.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
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<tr>
<td>17.</td>
<td>I enjoy social chit-chat.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
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<tr>
<td>18.</td>
<td>When I talk, it isn’t always easy for others to get a word in edgeways.</td>
<td>definitely agree</td>
<td>slightly agree</td>
<td>slightly disagree</td>
<td>definitely disagree</td>
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<td></td>
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<td>definitely agree</td>
<td>slightly agree</td>
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<td>19.</td>
<td>I am fascinated by numbers.</td>
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<td>20.</td>
<td>When I’m reading a story, I find it difficult to work out the</td>
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<td></td>
<td>characters’ intentions.</td>
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<td>21.</td>
<td>I don’t particularly enjoy reading fiction.</td>
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<td>22.</td>
<td>I find it hard to make new friends.</td>
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<td>23.</td>
<td>I notice patterns in things all the time.</td>
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<td>24.</td>
<td>I would rather go to the theatre than a museum.</td>
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<td>25.</td>
<td>It does not upset me if my daily routine is disturbed.</td>
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<td>26.</td>
<td>I frequently find that I don’t know how to keep a conversation</td>
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<td>going.</td>
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<td>27.</td>
<td>I find it easy to “read between the lines” when someone is</td>
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<td></td>
<td>talking to me.</td>
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<td>28.</td>
<td>I usually concentrate more on the whole picture, rather than</td>
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<td></td>
<td>the small details.</td>
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<td>29.</td>
<td>I am not very good at remembering phone numbers.</td>
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<td>30.</td>
<td>I don’t usually notice small changes in a situation, or a</td>
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<td>person’s appearance.</td>
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<td>31.</td>
<td>I know how to tell if someone listening to me is getting</td>
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<td></td>
<td>bored.</td>
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<td>32.</td>
<td>I find it easy to do more than one thing at once.</td>
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<td>33.</td>
<td>When I talk on the phone, I’m not sure when it’s my turn to</td>
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<td>speak.</td>
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<td>34.</td>
<td>I enjoy doing things spontaneously.</td>
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<td>35.</td>
<td>I am often the last to understand the point of a joke.</td>
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<tr>
<td>36.</td>
<td>I find it easy to work out what someone is thinking or feeling</td>
<td></td>
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<tr>
<td></td>
<td>just by looking at their face.</td>
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<tr>
<td>37.</td>
<td>If there is an interruption, I can switch back to what I was</td>
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<td></td>
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<tr>
<td></td>
<td>doing very quickly.</td>
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<td></td>
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</tbody>
</table>
38. I am good at social chit-chat.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

39. People often tell me that I keep going on and on about the same thing.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

40. When I was young, I used to enjoy playing games involving pretending with other children.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

41. I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

42. I find it difficult to imagine what it would be like to be someone else.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

43. I like to plan any activities I participate in carefully.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

44. I enjoy social occasions.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

45. I find it difficult to work out people’s intentions.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

46. New situations make me anxious.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

47. I enjoy meeting new people.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

48. I am a good diplomat.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

49. I am not very good at remembering people’s date of birth.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

50. I find it very easy to play games with children that involve pretending.  
   | definitely agree | slightly agree | slightly disagree | definitely disagree |

Developed by:  
The Autism Research Centre  
University of Cambridge
Appendix B: MRI Screening Form

MAGNETIC RESONANCE IMAGING (MRI) SCREENING FORM

Name: _______________________________________________________

Study: ___________________ SB Number ______________________

DOB: _____/_____/_______ Weight: ______ kg Height: ______

Gender: Male / Female Contact Phone Number: _____________

Contact Address: ____________________________________________

P/Code: ______

1. Have you ever had surgery or an operation? No / Yes
   If yes, please indicate the date and type of surgery _______________________

2. Have you ever had a diagnostic imaging study or examination (MRI, CT, X-ray)? No / Yes

3. Have you ever had:
   Epilepsy/Seizures: No / Yes
   Brain Infection: No / Yes
   Febrile Convulsions: No / Yes
   Psychiatric Disease: No / Yes
   Head Injury: No / Yes
   Other health issues: No / Yes

4. Do you currently have any neurological symptoms (e.g. weakness, double vision)? No / Yes

5. Are you on any medication? No / Yes

6. Are you allergic to any medication? No / Yes

7. Do you have any history of renal disease? No / Yes

For female patients:
8. Date of last menstrual period: _____/_____/_______ or Postmenopausal? No / Yes

9. Are you pregnant or experiencing a late menstrual period? No / Yes

Further details:

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________

_____________________________________________________________________
For Office Use Only

Status: Control / Patient Time In: ____:____ Time Out: ____:____
Notes: ________________________________________________________________

Certain implants, devices, or objects may interfere with the MR procedure. To help us to determine your suitability for an MRI scan and to ensure your safety, please complete the following checklist carefully.

Please indicate if you have any of the following:

<table>
<thead>
<tr>
<th>ITEM/DEVICE</th>
<th>NO</th>
<th>YES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneurysm Clips</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac Pacemaker / Defibrillator</td>
<td></td>
<td></td>
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<tr>
<td>Neuro-stimulation system</td>
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<td></td>
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<tr>
<td>Implanted electrical device</td>
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<tr>
<td>Vascular Surgery Clips</td>
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<tr>
<td>Artificial Heart Valve</td>
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<tr>
<td>Intra-ventricular or Spinal Shunt</td>
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<tr>
<td>Metallic Stent / Filter or Coil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical staples, metallic sutures or metallic plates</td>
<td></td>
<td></td>
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<tr>
<td>Eye Implant or Eye Operation</td>
<td></td>
<td></td>
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<tr>
<td>An injury to your eye involving metal fragments?</td>
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<tr>
<td>Cochlear or other Ear Implant</td>
<td></td>
<td></td>
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<tr>
<td>Orthopaedic Devices (screws/rods/pins/plates/nails etc.)</td>
<td></td>
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<tr>
<td>Any metallic fragment or foreign body</td>
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<tr>
<td>Dentures (false teeth)</td>
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<td></td>
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<tr>
<td>Hearing Aid (Please remove before entering MR room)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piercing Jewellery</td>
<td></td>
<td></td>
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<tr>
<td>Tattoo or Permanent Makeup</td>
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<td></td>
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<tr>
<td>Any other surgical procedures / operations / implants?</td>
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</tbody>
</table>

IMPORTANT INSTRUCTIONS
Before entering the MR room, you must remove all metallic objects including hearing aids, dentures, glasses, partial plates, mobile phones, pagers, watch, hairpins, safety pins, jewellery, body piercings, keys, coins, bank cards, magnetic strip cards, & pens.
If yes provide details: …………………………………
…………………………………………………………
…………………………………………………………

I confirm that the above information is correct to the best of my knowledge. I have read and understand the contents of this form and had the opportunity to ask questions regarding the information on this form and regarding the MR procedure that I am about to undergo.

Signed: 

Parent or Guardian if under 18: 

Witnessed: 

Date: _______
Appendix C: MRI Consent Form

MRI CONSENT FORM

Research studies carried out at BRI are designed to improve our knowledge and are not designed for clinical purposes.

After your scan a specialist will review the pictures; however this will not be done on the day of your study. You should be aware that sometimes even in completely healthy people, minor abnormalities are found. On the other hand, because the pictures are taken for a specific research purpose, not all abnormalities are necessarily seen. On extremely rare occasions, we might find an abnormality that is significant and which may need to be investigated further. If a significant abnormality is found, we will contact the researcher directly involved in your study.

Although such a finding is extremely unlikely, please take the time to consider carefully what it would mean to you. It would be entirely your choice as to what you might do with any such information. However, knowledge of an abnormality may affect your ability to do such things as work in certain professions, obtain life or health insurance, etc. If you do not want to know, then you are under no obligation to participate in this part of the study.

I confirm that I have read and understand the above information and that I have had the opportunity to ask questions. I confirm that I agree to have an MRI scan as part of this research study.

Signed: __________________________ Date: __________________________

Parent or Guardian if under 18: _______________ Date: _______________

Witnessed: __________________________ Date: ______________________

I have agreed to have an MRI scan as part of this specific research study, but I am aware that the data may also be useful for other studies in the future.

I therefore **AGREE / DO NOT AGREE** (please circle as appropriate) to the data being used for further research purposes.

Signed: __________________________ Date: __________________________

Parent or Guardian if under 18: __________________ Date: _______________

Witnessed: __________________ Date: __________________________