Structural and Functional Aspects of Brain Development in Children with an Autism Spectrum Disorder (ASD)

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Graduate Department of Psychology

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Abstract

Research suggests that brain growth follows an abnormal trajectory in children with autism spectrum disorders (ASD). A better understanding of when and how patterns of brain development diverge from that seen in typically developing children could yield insight into the etiology of the disorder, and resulting symptomatology. To investigate this hypothesis, three studies examined the relation between structural and functional brain measures and age in a group of children with an ASD, aged 6 to 14 years. Age by group interactions were found in all three studies, providing further evidence that brain development may follow an atypical trajectory in ASD. Study 1: Differences in the relation between structural indices and age were found in grey matter volume, surface area and thickness, as well as in cortical thickness of specific regions in the left inferior frontal gyrus (BA 44) and left precuneus. These measures of grey matter structure generally decreased with age in the ASD children, compared to little or no change with age in the typically developing children. Study 2: Differences in the relation between age and measures of longitudinal, radial and mean diffusivity were found in frontal, long distant, interhemispheric and posterior white matter tracts; diffusivity decreased with age in the typically developing group, but showed little or no change in the ASD group. Study 3: Differences in the relation between BOLD activation on a set-shifting task and age were found in
brain regions important for cognitive flexibility, such as areas of prefrontal, right insula and parietal cortex. These effects were mainly due to decreasing activation with age for the ASD group, but increasing or no age-related change in the TD group. The findings of these three studies provide converging evidence in support of an hypothesis of dysregulated brain development in this population, which could have significant, compounding effects on the development of neural connectivity, and contribute to atypical cognitive development in children with ASD.
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Appendix 1: Supplemental Material for Chapter 3

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1 General introduction

1.1 Autism spectrum disorder (ASD)

Autism spectrum disorder (ASD) refers to a syndrome of developmental impairments in communication and social skills, in combination with rigid, repetitive behaviour. The spectrum is thought to include autistic disorder, Asperger’s disorder, and Pervasive Developmental Disorder not otherwise specified (PDD-NOS), as these disorders are described in the fourth edition of the Diagnostic and Statistical Manual (Diagnostic and statistical manual of mental disorders: DSM-IV. 1994). However, draft revisions to the fifth edition of the DSM propose the merger of these three diagnostic categories together into a single Autistic Spectrum Disorder, due to a lack of consistent behavioural distinctions among them (American Psychiatric Association, 2010). This is supported by a lack of evidence for neurobiological distinctions among these categories as well (e.g., Via, Radua, Cardoner, Happé, & Mataix-Cols, 2011).

Epidemiological studies report a dramatic increase in the prevalence of ASD over the last few decades. Reports estimate the prevalence of ASD at 1 in 150, to as high as 1 in 90 individuals (King & Bearman, 2009), with males being affected three or more times more often than females (Chakrabarti & Fombonne, 2001). Broadening of diagnostic criteria to include the higher-functioning individuals, as well as heightened awareness likely accounts for the majority of this increase (Fombonne, 2002; Gernsbacher, Dawson, & Goldsmith, 2005), though some contribution of a true rise over time cannot be conclusively ruled out.

The term “autism” (from the Greek “autos”, meaning “self”) was first used in 1911 by Bleuler (1911/1950), to describe a group of individuals with schizophrenia who had lost contact with reality and were inwardly, or self, focused. The term was then adopted by Leo Kanner in 1943, and Hans Asperger in 1944, who independently published the first descriptions of children without a diagnosis of schizophrenia but displaying the now well-known triad of ASD symptoms: impairment in social relationships, abnormal language and restricted and repetitive interests. Kanner (1943) described 11 children displaying “extreme autistic aloneness” (p.242), having an “inability to relate themselves…to people and situations” (p. 242), abnormalities in their language such as delays in acquisition, echolalia, and pronoun reversals, and an “obsessive desire for the maintenance of sameness” (p. 245), but normal cognitive abilities. Asperger (1944)
described a similar but less impaired group of 4 children, to whom he gave a diagnosis of “autistic psychopathy”. These children also had social impairments, notably difficulties with eye contact and emotional expression. In contrast to Kanner’s description, these were described as having typical language abilities (i.e., age appropriate vocabulary and grammar). However, they still demonstrated impairment in communication in the form of poor conversational abilities, atypical flow of speech, and often had abnormalities in the volume and tone of their speech. He also described a tendency to become extremely preoccupied with narrow topics of interest. Children who fit this latter description have since been given a diagnostic label of “Asperger’s disorder”, as described in the DSM-IV (Diagnostic and statistical manual of mental disorders: DSM-IV.1994).

Early theories proposed that autism was caused by emotional disturbance due to negative early attachment experiences, specifically from cold and rejecting parents (usually mothers) (Kanner, 1943; Bettelheim, 1967). In fact, treatments for autism aimed at helping parents become less rejecting toward their children continued until the mid 1970s (Klinger, Dawson & Renner, 2003). However, this proposed etiology was not supported by any empirical research conducted in the 1970s and 80s, and was gradually rejected. In 1964, a neurological cause for autism was first proposed (Rimland, 1964), marking the beginning of a significant research focus in this area. After decades of work it is now well accepted that ASD is a genetic disorder of prenatal and postnatal brain development (DiCicco-Bloom et al., 2006). However, the specific neurodevelopmental mechanisms, as well as the functional and cognitive sequelae (ultimately leading to the known behavioural symptoms), remain the subject of intense study and debate. No unifying pathophysiological theory of autism or biologic diagnostic test has yet been developed. Discovery of underlying biological markers, and their functional implications, will be critical to the development of more accurate and earlier diagnosis, as well as a better understanding of the phenotypic heterogeneity of ASD, as we move forward into the next era of research and clinical practice.

In what follows in this chapter, I will first review the findings from investigations of neurological markers of ASD, in structures across the brain as well as studies of grey and white matter (Section 1.2). I will then review typical early brain development and an important neurodevelopmental theory of ASD that has arisen from the aforementioned investigations of
neurological markers (Section 1.3). Finally, I will review the major cognitive models of ASD (Section 1.4) and discuss underlying brain functional atypicalities (Section 1.5).

1.2 Structural neuroimaging in autism spectrum disorder

1.2.1 Structural magnetic resonance imaging (MRI) studies

At first glance, a review of the structural and volumetric magnetic resonance imaging (MRI) data for ASD may appear plagued by variability and lack of consensus. However, patterns are emerging. One of the most consistent results has been the repeated finding of abnormalities in overall brain volume. Evidence for this comes from studies of head circumference, an indirect measure of brain volume, as well as MRI studies. Overall, brain volume increases appear to be more pronounced in younger children with ASD and peak around 2-4 years of age (Aylward, Minshew, Field, Sparks, & Singh, 2002; Carper, Moses, Tigue, & Courchesne, 2002; Carper & Courchesne, 2005; Courchesne, 2002; Hardan, Minshew, Mallikarjuk, & Keshavan, 2001; Sparks et al., 2002; and see Redcay & Courchesne, 2005 and Amaral, Schumann, & Nordahl, 2008 for reviews). This early acceleration in brain growth may be followed by a plateau (Courchesne et al., 2001; Redcay & Courchesne, 2005), during which typical growth patterns converge and group differences disappear, but this has not yet been confirmed by larger longitudinal studies. Results from a number of brain volume studies are shown in Figure 1.1.

The enlargement in volume appears to involve both grey and white matter, although some studies have suggested that white matter may be more involved (Carper et al., 2002; Courchesne et al., 2001; Herbert et al., 2004). Some studies have also shown that the frontal lobes may be particularly affected in this volumetric increase (e.g., Carper et al., 2002; Carper & Courchesne, 2005), but again, this has not been shown in other studies (e.g., Piven, Arndt, Bailey, & Andreasen, 1996).
Many studies have also further identified particular regions of the brain with volumetric abnormalities in individuals with ASD. Although consistency between studies is lacking, evidence appears to be accumulating for abnormalities in particular regions, such as the cerebellum (e.g., Courchesne, Yeung-Courchesne, Press, Hesselink, & Jernigan, 1988; Stanfield et al., 2008; Rojas et al., 2006), the corpus callosum (e.g., Piven, Bailey, Ranson, & Arndt, 1997; Stanfield et al., 2008, Casanova et al., 2011), the amygdalae (e.g., Mosconi et al., 2009; Schumann et al., 2004; Schumann, Barnes, Lord, & Courchesne, 2009; Sparks et al., 2002), basal ganglia (Hollander et al., 2005; Sears et al., 1999, Stanfield et al., 2008), and hippocampi (e.g., Bauman & Kemper, 2005; Saitoh et al., 2001). Individual studies have also found abnormalities in the volumes of the thalamus (Tsatsanis et al., 2003) and brainstem (Rodier, 2002).

The breadth and heterogeneity of these findings suggest that the neural correlates of autism are widely distributed throughout the brain and affect both grey and white matter. Newer imaging methods allow for more sophisticated characterization of grey and white matter structure. Cortical grey matter can be further analyzed in terms of its subcomponents of cortical thickness and surface area which can be derived from MRI data, and for white matter, studies using
diffusion tensor imaging (DTI) are providing a new level of analysis for white matter structure, both of which I review below.

1.2.2 Cortical grey matter studies

MRI data can now be used to derive not only measurements of brain volume, but also of its subcomponent measures of cortical thickness and surface area. These two measures are thought to depend on different maturational processes. It has been hypothesized that cortical thickness reflects dendritic arborisation and pruning within grey matter (Huttenlocher, 1990) or changes in myelination at the interface of grey and white matter (Sowell et al., 2004), whereas surface area is dependent on division of progenitor cells in the periventricular area during embryogenesis (Chenn & Walsh, 2002) and varies with degree of cortical folding/gyrification. Investigation of differences in these measures of cortical grey matter may provide important indications of very early neuroanatomical developmental events in the ASD population.

Initial reports of cortical thickness have shown that cortical thickness is increased in ASD relative to controls in adult samples (e.g., Bailey et al., 1998; Hutsler, Love, & Zhang, 2007) but recent reports have been more variable. A study of adults by Hadjikhani, Joseph, Snyder and Tager-Flusberg (2006) found evidence of thinner cortex in areas important for social cognition, and a study of adolescents by Chung et al. (2005), using a vertex-by-vertex analysis allowing for regional specificity of CT differences also showed evidence of areas of thinner cortex in the ASD group, in right inferior orbitofrontal cortex, left superior temporal sulcus, and left occipitotemporal gyrus. A recent report by Wallace, Coleman, Pascalis, and Bailey (2006) in a group of young adults with ASD also found gyral and vertex-based results of thinner cortex in some left temporal and parietal areas (superior temporal sulcus, inferior temporal, postcentral/superior parietal and supramarginal gyri). However, Hyde, Samson, Evans, and Mottron (2010), also using a vertex-based approach, found evidence of increased cortical thickness in an adult ASD group relative to a matched control group in regions from all four lobes. A small number of regions had thinner cortex in the autism group (the pre- and post-central gyri, and the paracentral gyrus); however, the majority of findings were in the direction of increased cortical thickness (Hyde et al., 2010). In the study by Hutsler et al. (2007), the difference in cortical thickness between matched pairs tended to decrease with age, suggesting that cortical thickness increases were more pronounced at younger ages, although this relation
failed to reach significance. The study by Wallace et al. (2006) found evidence of an age by group interaction in the left fusiform/inferior temporal cortex, where ASD participants were showing greater thinning with age, to a greater degree than the typically developing group.

Studies of cortical thickness in children with ASD, although only two have been reported, have tended to show evidence of increased cortical thickness. Using measures of average CT across hemispheres and lobes, (Hardan, Muddasani, Vemulapalli, Keshavan, & Minshew, 2006) found no differences in overall brain volume between the two groups, but significantly thicker cortex in the whole brain overall for the boys with autism, with similar findings in the temporal and parietal lobes. These results are represented in Figure 1.2 below.

![Cortical Thickness Differences by Lobe](image)

**Figure 1.2** Cortical thickness differences reported by Hardan et al. (2006) between children with ASD and typically developing children. (*p*<0.05, **p**<0.01; error bars represent one standard deviation).

In a follow up report, Hardan, Libove, Keshavan, Melhem and Minshew (2009) showed evidence of decreases in total grey matter and cortical thickness with time for the ASD group relative to the control group. In contrast to the Hardan (2009) study, in a sample ranging from 10 to 45 years of age (thus including some children but mostly adults), Raznahan et al. (2010) found that in regions showing an age by group interaction, there was no relation between age and changes in volume or cortical thickness in the ASD group, compared to significant decreases in
cortical thickness and volume with age in the control group. Raznahan et al. (2010) reported that in their results cortical thickness in children with ASD was decreased relative to typically developing children, and increased relative to the ASD group at later ages. These latter data are inconsistent with the many studies showing early brain overgrowth in children with ASD, with a relative decrease in brain size later in childhood.

Considerably fewer studies have examined changes in surface area in ASD. Surface area is determined by cortical folding, and there is some evidence that this may be altered in ASD. In a preliminary study including children and adults with ASD, Hardan, Jou, Keshavan, Varma and Minshew (2004) showed a greater gyrification index in the left frontal lobe for children and adolescents with ASD, but not in adults, as well as decreased cortical folding with age for the ASD participants relative to control participants. Raznahan et al. (2010) reported that cortical surface area showed minimal changes with age and this pattern did not differ between the ASD and typically developing control group. Sulcal location (Levitt et al., 2003) and sulcal depth (Nordahl et al., 2007) may also be atypical in children with ASD.

Abnormal gyrification may be directly linked to the previously reported increases in brain volume (Armstrong, Schleicher, Omran, Curtis, & Zilles, 1995). Importantly, these changes in volume and gyrification have a profound impact on connectivity. Increased gyrification causes a decrease in gyral width, which consequently reduces the area of grey matter, known as the “gyral window”, that is available for contact with the white matter below (Prothero & Sundsten, 1984). Therefore, it is theorized that larger brains may result in less connectivity between regions of cortex.

Evidence has also emerged for abnormalities in grey matter on a microstructural level. Studies by Casanova and colleagues have demonstrated abnormalities in ASD in the spacing and size of minicolumns, a primary unit of organization within the neocortex (Casanova, Buxhoeveden, Switala, & Roy, 2002). Specifically, there were more minicolumns in the frontal and temporal lobes of individuals with ASD, and they were smaller and less compact in their arrangement (Casanova et al., 2002). Minicolumns are a building block of cortical interconnectivity. It is estimated that each minicolumn is connected to $10^3$ others (Casanova, 2004). Casanova and colleagues propose that a pathological increase in minicolumns within the brain of individuals
with autism may explain the increased brain size and the resulting changes in gyrification and connectivity (Casanova, 2006).

Clearly further work is required in these cortical measures to better determine the neurodevelopmental patterns in ASD and how they differ from typically developing children.

1.2.3 Diffusion tensor imaging white matter studies

DTI is a magnetic resonance imaging technique that measures the diffusion of water within tissue (Basser, Matiello & LeBihan, 1994a, Basser, Matiello, & LeBihan, 1994b, Basser, Matiello & LeBihan, 1996). White matter tracts have more organized microstructure, impeding diffusion across the tracts but not along the tracts resulting in an anisotropic diffusion pattern. Comparatively, in gray matter water diffusion is more uniform across directions (an isotropic diffusion pattern). The tensor model describes this anisotropy as an ellipsoid, with three eigenvectors describing the orientation of the radii of ellipsoid and three eigenvalues describing the lengths of the radii (Basser, Matiello, & LeBihan, 1994b). The first eigenvalue (Dmax), or longitudinal diffusivity, is the magnitude of diffusion along the primary eigenvector, which is assumed to run parallel to the fibre tract. Reduced Dmax is thought to be related to reduced axonal integrity, a complex construct that may include accumulation of cellular debris, disordered microtubule arrangement, aggregation of microfilaments, cellular swelling and decreased axonal transport (Sun, Liang, Trinkaus, Cross, Armstrong & Song, 2006). The two other smaller eigenvectors are assumed to run perpendicular to the main direction of the white matter tracts, and their eigenvalues can be averaged together to represent radial diffusivity (Drad). Increased radial diffusivity is thought to be a marker of reduced myelin integrity (Song, Sun, Ramsbottom, Chang, Russell, Cross, 2002; Klawiter et al., 2011). Mean diffusivity (MD) is the average of these three eigenvalues. The calculation of fractional anisotropy (FA) is related to the differences among the three eigenvalues and characterizes the anisotropy on a scale from 0 to 1, with larger numbers reflecting greater deviation from a sphere; where diffusion is fully isotropic, FA = 0. Over childhood MD, Dmax and Drad decrease and FA increases with age and normal white matter development (Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008; Snook, Paulson, Roy, Phillips, & Beaulieu, 2005). Thus, several measures can be extracted from DTI neuroimaging studies, which will contribute to a fuller understanding of differences in white matter development in ASD.
FA has been the measure of choice in most previous papers examining differences in DTI between individuals with ASD and a typically developing control group. Lower FA in a group comparison has typically been interpreted to reflect decreased organization and coherence within fibre tracts. Although locations have been variable (orbitofrontal, medial prefrontal, temporal lobe, corpus callosum, cingulate cortex, arcuate fasciculus, ILF, uncinate fasciculus, cerebellar outflow tracts, internal capsule), and different techniques have been used, including tractography and voxel-based techniques, the majority of studies have found evidence of reduced FA in children (Barnea-Goraly et al., 2004; Barnea-Goraly, Lotspeich, & Reiss, 2010; Brito et al., 2009; Cheung et al., 2009; Ke et al., 2009; Sahyoun, Belliveau, Soulières, Schwartz, & Mody, 2010; Sundaram et al., 2008) and adults with ASD (Catani et al., 2008; Keller, Kana, & Just, 2007; Lee et al., 2007; Pardini et al., 2009; Thakkar et al., 2008). However, at least a few recent studies have found evidence of regions of increased FA in ASD, in samples of young children (Ben Bashat et al., 2007) and adolescents (Cheng et al., 2010). Despite the variability in brain areas reported, the large number of groups finding evidence of abnormalities in white matter measures itself suggests that there is credence to these findings. However, because FA is an aggregate and non-specific measure, investigation of the individual eigenvalues are needed to better interpret any differences in DTI that may exist between clinical and control groups. Recent studies have begun to report the sources of these differences in radial and axial diffusivity. Two recent reports showed have implicated abnormalities in radial diffusivity, with one study reporting increased mean diffusivity in the arcuate fasciculus in adolescents with ASD, mainly due to increases in radial diffusivity, implicating the myelin component of white matter in ASD pathophysiology (Fletcher et al., 2010) and another reporting increases in children with ASD in radial diffusivity in the whole brain, the corpus callosum and internal capsule (Shukla, Keehn, Lincoln, & Müller, 2010). However, at least one study has highlighted widespread decreases in FA within frontal parietal and temporal lobes, driven by decreases in axial but not radial diffusivity (Barnea-Goraly et al., 2010). A consensus has yet to emerge on the significance of abnormalities in axial versus radial diffusivity in this population, and more studies assessing these measures are necessary to further our understanding of the white matter deficits in ASD.
### 1.3 Typical brain development and the growth dysregulation hypothesis of ASD

Early brain development is a balance of simultaneous, ongoing progressive and regressive events. Progressive events, which characterize the early postnatal period, include neurogenesis, axon guidance, growth of synapses, fibre tracts and dendritic arborization, while regressive events include apoptosis, axonal pruning, and elimination of synapses, and may be more related to experience (Acosta, Gallo, & Batshaw, 2002; Huttenlocher & Dabholkar, 1997). Each of these progressive and regressive events occurs not in isolation, but as the outcome of a series of preceding events, and the basis for subsequent ones, creating a complex succession. This succession of events unfolds, in a delicate balance with experience, along the developmental timecourse.

The non-invasive technology of magnetic resonance imaging (MRI) has allowed for significant progress in the study of the anatomy and physiology of the developing brain over the last two decades. Initial studies focused on individual brain components averaged across large age ranges, but recent studies have involved large groups of children, followed longitudinally, to carefully examine and characterize trajectories of brain development over time. In a longitudinal study of 829 scans of 387 individuals, ages 3-27 years, it was shown that total cerebral volume follows an inverted U-shaped trajectory, peaking at 10.5 years for females and 14.5 years for males (R. K. Lenroot et al., 2007) (Figure 1.3). The cerebellum followed a similar U-shaped curve, with peak volume occurring at 11.3 years of age for females and 15.6 years in males. This developmental maturation occurred in the cerebellar hemispheres, but not in the evolutionarily older cerebellar vermis (Tiemeier et al., 2010). White matter volume generally increases over childhood and adolescence (Lenroot et al., 2007) (Figure 1.3). It is well known that increased myelination of axons causes dramatic increases in the speed at which neuronal transmission can occur, and is therefore critical for structural formation of connecting pathways in the brain, but recent findings suggest that myelin also modulates the timing and synchrony of neuronal firing patterns that create functional networks in the brain (Fields & Stevens-Graham, 2002). Although white matter and grey matter do not exist in isolation from each other, they do appear to develop differently. In contrast to the increases in white matter, gray matter volumes follow an inverted U-shaped trajectory (Figure 1.3).
Figure 1.3 Mean volumes for total brain, grey matter, and white matter for males (blue lines) and females (red lines). Total brain volume and grey matter show the inverted u-shaped curve, whereas white matter steadily increases. Adapted from Lenroot et al. (2007). Reprinted with permission from Elsevier.

However, there is regional differentiation in development within grey matter. Peak grey matter volume is reached first in primary sensory and motor areas, and latest in higher-order association cortices such as dorsolateral prefrontal cortex, inferior parietal cortex, and superior temporal gyrus (Gogtay et al., 2004). Differences in regional maturation were also found in a study of changes in cortical thickness from 764 scans acquired from 375 typically developing children and young adults aged from 3.5 to 33 years old, showing markedly different patterns of cortical growth across different brain regions (Shaw et al., 2008). In general, lower-order allocortical regions, such as limbic areas, showed predominantly simple and linear growth trajectories, where peak thickness is achieved early in life, followed by a gradual decline into adulthood. In contrast, high-order association areas of the cortex generally have more complex developmental trajectories. Cubic trajectories, with a period of initial childhood increase in cortical thickness followed by adolescent decline and stabilization in adulthood, are seen throughout most of lateral frontal, lateral temporal, parietal and occipital cortex. Quadratic trajectories are seen in insula and anterior cingulate cortex, where growth shows the increase in childhood and adolescence, peaking only in the late teenage years. These areas are critically involved in social emotional tasks and higher order cognitive functions. Simple linear trajectories are seen largely in areas on the inferior aspect of the brain, such as posterior orbitofrontal and frontal operculum, portions of piriform cortex, medial temporal cortex, subgenual cingulum, and medial occipitotemporal cortex (Shaw et al., 2008) (Figure 1.4). Synaptic pruning and/or myelination at the border of grey and white matter may be driving this cortical thinning/decrease in cortical volume (Sowell, Delis, Stiles, & Jernigan, 2001).
A representative medial section of the brain showing different developmental trajectories depending on brain region. Trajectories of cortical thickness are cubic (shown in red) throughout most of lateral frontal, lateral temporal, parietal and occipital cortex. Quadratic curves (shown in green) are seen in insula and anterior cingulate cortex. Linear trajectories (shown in blue) are seen in posterior orbitofrontal and frontal operculum, portions of piriform cortex, medial temporal cortex, subgenual cingulum, and medial occipitotemporal cortex (Shaw et al., 2008).

It is now thought that the shape of the age-related trajectories may be more related to brain function than absolute size at any one point (Giedd & Rapoport, 2010). For example, a longitudinal study of 692 scans of 307 typically developing individuals showed that age by cortical thickness developmental curves were more related to IQ than differences in cortical thickness at age 20 (Shaw et al., 2006). Developmental trajectories are also better able to discriminate between gender than static measures, as previously mentioned (Lenroot et al., 2007).

The structural evidence summarized in section 1.2.1 shows that in ASD, the trajectory of brain growth during this critical period is abnormal, with brain overgrowth early in life, possibly due to dysregulation of regressive events in particular (Akshoomoff, Pierce, & Courchesne, 2002). Based on these findings, a “growth dysregulation hypothesis” of ASD has emerged (Akshoomoff et al., 2002; Acosta & Pearl, 2004). The consequences of such dysregulated growth early in life would be widespread and pervasive throughout the brain, resulting in abnormalities in neuronal migration, cortical organization, and myelination, and subsequently the reported abnormalities in
cortical thickness, organization of white matter, and atypicalities in gyrification patterns as summarized above. This would then have profound consequences on early functional development of systems for various neurobehavioural domains. The early overgrowth may also be followed by a period of deceleration or even arrest in brain growth (Redcay & Courchesne, 2005), and thus the growth dysregulation in ASD may also include a lack of normal acceleration in growth that usually occurs in adolescence. Brain size is “normalized”, but this has occurred in an atypical way. This period of maturation is associated with the emergence of a second phase of higher-order abilities, particularly frontal lobe functions (Casey, Giedd, & Thomas, 2000).

1.4 Cognitive theories of autism spectrum disorder

There are three main cognitive theories of ASD: the Theory of Mind (ToM) hypothesis (Baron-Cohen, Leslie, & Frith, 1985), the Weak Central Coherence theory (Frith, 1985; Happe, 1999), and the Executive Dysfunction theory (Hughes, Russell, & Robbins, 1994; S. Ozonoff & Jensen, 1999). This thesis includes work that addresses only the latter of the three theories, which will be reviewed in detail below.

1.4.1 Theory of Mind (ToM) hypothesis

A number of cognitive theories have been proposed to explain the relation between brain and behaviour in autism. The first well-known theory is the Theory of Mind (ToM) hypothesis (Baron-Cohen, Leslie, & Frith, 1985). This theory proposes that a neurologically based deficit in social processing, primarily characterized by an inability to recognize or share other’s mental states (i.e., have a ‘theory of mind’), is responsible for the social communication impairments seen in ASD. This theory is sometimes also referred to as “mindblindness” or “mentalizing failure” (Hill & Frith, 2003) and has been investigated quite extensively. The first study to test this hypothesis was published in 1985 by Baron-Cohen et al., using what is now a well-known “false-belief” task. Children are shown two dolls, named Sally and Ann. Sally places a marble in her basket, and leaves. While she is gone, Ann moves Sally’s marble to her own basket. When Sally returns, she wants to play with her marble. Children were asked, “Where will Sally look for her marble?” The correct answer is that Sally will look for the marble in her own basket, even though it will not actually be there. Eighty percent of children with autism failed to answer this question correctly, and stated that Sally would look for her marble in Ann’s basket. They were not able to take the perspective of Sally’s mental state, to reason that she would look for the
marble where *she* left it, and not where it actually was. This was in contrast to a comparison group of children with Down Syndrome, of matched mental age, who were able to pass the test question at a rate of 86% (Baron-Cohen et al., 1985). Children and adults with autism have also been shown to have difficulties with other mental state functions such as pretence, irony, non-literal language and deception. Primarily assessed using tests of story understanding, individuals with autism have shown to lack an intuitive understanding about motivation of characters, despite intact understanding of cause-and-effect reasoning about the stories (see Hill & Frith, 2003, for a review).

Overall, behavioural evidence for a deficit in mentalizing in autism is fairly strong, and it can account fairly well for the core impairment in social communication in ASD. However, it is not a comprehensive theory of ASD because it does not account as well for other non-social symptoms or behavioural impairments seen in ASD, such as repetitive behaviour.

### 1.4.2 Weak Central Coherence (WCC) theory

Individuals with ASD also display a number of non-social behavioural symptoms, such as repetitive behaviour and restricted interests, as well as an uneven pattern of intelligence comprised of peaks and valleys (Hill & Frith, 2003). A second well-established cognitive theory of ASD that aims to explain the latter observation is known as Weak Central Coherence (WCC; Happé, 1999). Central coherence is the term coined by Frith (1989) for the tendency of typically developing individuals to process incoming information coherently; that is, pulling information together for higher-level meaning, often at the expense of memory for detail. Evidence for this comes from global level processing advantages or intereference seen in typically developing participants on tasks such as story recall (recalling gist better than details) and some perceptual tasks (e.g., difficulty on The Embedded Figures Test, where a small part must be located within a global picture (EFT; Witkin et al., 1971). The WCC model proposes that individuals with ASD, in contrast to this typical tendency, process information according to a more bottom-up, detail-focused processing style, at the expense of processing the overall gestalt or context. Proponents of this theory propose weak central coherence as the explanation for superiority in performance for individuals with ASD on tests that benefit from detailed, piecemeal processing, such as the block design subtest on the Wechsler intelligence scales (Shah & Frith, 1993), or the EFT (Shah & Frith, 1983), as well as other so-called savant abilities in highly specific areas (e.g., jigsaw
puzzles, perfect pitch, mnemonic skills; Happé, 1999). They also tend not to show the typical benefit from semantic or contextual meaning on memory tests, or interference from prior semantic context (see Happé, 1999, for a review). Individuals with ASD could be less influenced by the global context or gestalt, or may have difficulties integrating local information into the overall gestalt. Savant abilities in ASD could arise from an obsessive interest in small details leading to a highly developed skill in a very specific area. However, it is of note that it has also been proposed that this pattern of performance could be due to an enhancement of discrimination for the individual elements of a stimulus, rather than a failure of integration (e.g., Mottron, Peretz, & Ménard, 2000).

Together, the ToM and WCC theories can explain a number of the different social and non-social symptoms of ASD. However, a noted weakness of both ToM and WCC theories is an inability to explain the restricted and repetitive behaviour that is also an important hallmark of ASD.

### 1.4.3 Executive Dysfunction theory

The last of the major cognitive theories of ASD aims to fill the gap left by the other cognitive theories, and provide a cognitive basis for the restricted and repetitive behaviour symptoms seen in ASD, termed the “executive dysfunction” theory of autism (Hughes, Russell, & Robbins, 1994; S. Ozonoff & Jensen, 1999). Executive function (EF) is an umbrella term that refers to cognitive functions important for goal-directed activity such as planning, working memory, inhibition and cognitive flexibility, as well as the initiation and monitoring of action. This theory grew out of observation that individuals with autism often exhibited difficulties similar to patients with frontal lobe damage. Specifically, individuals with ASD have been shown to have impairments in cognitive flexibility, planning and, to a lesser extent, inhibition (Hill, 2004).

Planning is a complex, dynamic operation in which a sequence of planned actions must be constantly monitored, re-evaluated and updated. Individuals with ASD have been shown to be impaired on behavioural tests of planning, such as the Tower of Hanoi (Ozonoff & Jensen, 1999) or the complex problems of the Stockings of Cambridge task (Hughes et al., 1994). These tasks require participants to carry out a sequence of planned actions, using real or computer generated manipulatives, which must be moved, one by one, from a predetermined starting state to a given goal state (disks of different diameters arranged in ascending size in the Tower of Hanoi, or balls placed in a column in a specific colour order in the Stockings task). Efficiency of planning can
be measured by the number of actions a participant makes to achieve the goal state. A well-planned solution will take the minimum number of moves possible, while a poorly planned solution will include many extraneous moves that do not contribute to the overall solution.

Cognitive flexibility is the ability to shift to a different strategy or action according to changes in a situation, in order to obtain a goal or solve a problem. A few tasks have been used to assess cognitive flexibility behaviourally in ASD, with the most commonly used task being the Wisconsin Card Sorting Task (WCST). On this task, participants must sort a series of cards into categories based on one of three different strategies: colour, shape or number. The sorting rule is never given explicitly but must be deduced using hypothesis testing and feedback from the examiner. After a series of consecutive correct sorts according to the rule (e.g. colour), the rule is changed, and participants must change their sorting behaviour accordingly to deduce the new rule. A number of studies have shown impaired performance for individuals with ASD on the WCST (e.g., Ozonoff & Jensen, 1999; Lopez, Lincoln, Ozonoff, & Lai, 2005; but see Robinson, Goddard, Dritschel, Wisley, & Howlin, 2009 for an exception).

Evidence for cognitive flexibility impairment in ASD also comes from performance on the Cambridge Neuropsychological Test Automated Battery (CANTAB) Intradimensional-Extradimensional (ID-ED) Shift Task, as developed by Dias, Robbins, & Roberts (1996). On this task, participants use feedback to learn to discriminate between stimuli on the basis of a single dimension (e.g. shape) and choose the correct shape on successive trials. Once they have established the discrimination rule, the stimuli change and a new rule must be deduced again using feedback. The new discrimination may pertain either to the same dimension as before (e.g., a new, unlearned shape becomes “correct”) which is referred to as an intradimensional (ID) shift, or to a new, previously ignored dimension (e.g., a line pattern overlaid on top of the shapes), which is referred to as an extradimensional (ED) shift. These ED attentional shifts are thought to demand greater cognitive flexibility in order to successfully make the switch. Some studies, including the largest on autism study to date, have shown that individuals with ASD were impaired on ED shifts, but performed similarly to controls on ID shifts (Ozonoff et al., 2004; Hughes et al., 1994), however, other studies have failed to replicate this finding (e.g., Corbett, Constantine, Hendren, Rocke, & Ozonoff, 2009).
Cognitive flexibility has also been examined in ASD using a number of other tasks such as the modified card sorting task (MCST), the Trail Making Test (TMT), the Children’s Color Trail Test (CCTT), the Behavioural Assessment of the Dysexecutive Syndrome (BADS) Switch task, the Dellis-Kaplan Executive Function System (D-KEFS) Colour Word task. Evidence from these studies has been mixed, with some, but not all studies finding differences (Corbett et al., 2009; Lopez et al. 2005; Barnard, Muldoon, Hasan, O'Brien, & Stewart, 2008; Goldstein, Johnson, & Minshew, 2001; Goldstein, Minshew, Allen, & Seaton, 2002; Hill & Bird, 2006; Minshew, Meyer, & Goldstein, 2002).

Studies of inhibition in individuals with ASD have yielded mixed results. Participants with ASD have shown impaired performance on certain tasks, such as switching to a “go” response for a given stimulus previously associated with a “no-go” response (Ozonoff & Strayer, 1997), or a task which involves overriding a prepotent motor response (pointing away rather than toward a target object to obtain a reward) (Russell, Mauthner, Sharpe & Tidswell, 1991). However, in other studies participants with ASD have shown intact performance on other traditional tests of inhibition such as a Stroop task (e.g., Ozonoff & Jensen, 1999). Thus it appears that problems with inhibition are evident on some tasks, but they are not present across the board in individuals with ASD, or may be sensitive to group demographics or protocols used.

The “executive dysfunction” theory of autism suggests that these underlying deficits in executive function result in a tendency to get “stuck” in set (Hill, 2004). This tendency to get “stuck” is the underlying cognitive basis of the behavioural rigidity, perseveration and repetitive behaviour symptoms that are one of the definitive hallmarks of ASD. This theory therefore posits a direct relationship between executive function impairment and repetitive behaviour symptoms in ASD. The theory is limited by the fact that executive dysfunction is obviously not unique to autism, appearing in many other disorders, and also that it may not be universal within individuals with autism, despite displaying symptoms of restricted interests and repetitive behaviour by diagnostic definition. Some individuals with ASD and normal IQ may perform well on tests of executive function in some studies (Russell & Hill, 2001). Thus, it is limited in use as a diagnostic marker, but may still be helpful in understanding the cognitive basis for impairments seen in many cases of ASD. The cognitive theories are not necessarily mutually exclusive, but may together provide a useful framework for understanding the cognitive impairments seen in ASD, and provide hypotheses for further investigations, such as neuroimaging studies.
1.5 Functional neuroimaging in autism spectrum disorder

1.5.1 Functional magnetic resonance imaging of core symptom domains

A large number of functional neuroimaging studies have been conducted in ASD. The first phase of these studies focused on brain-behaviour relations of individual functions, within core symptom domains. For example, studies have shown neural abnormalities on tasks of social cognition such as face processing (Ashwin, Baron-Cohen, Wheelwright, O'Riordan, & Bullmore, 2007; Bookheimer, Wang, Scott, Sigman, & Dapretto, 2008; Corbett et al., 2009; Greimel et al., 2010; Koshino et al., 2008; Pierce & Redcay, 2008; Schultz et al., 2000), theory of mind (Castelli, Frith, Happé, & Frith, 2002; Wang, Lee, Sigman, & Dapretto, 2007), and imitation (Iacoboni & Dapretto, 2006; Williams et al., 2006) and tasks of language such as sentence comprehension (Just, Cherkassky, Keller, & Minshew, 2004), semantic processing of words (Harris et al., 2006), reading phrases for response-naming (Knaus, Silver, Lindgren, Hadjikhani, & Tager-Flusberg, 2008), verbal fluency (Kleinhaus, Müller, Cohen, & Courchesne, 2008) and processing of sentences with high and low imagery (Kana, Keller, Cherkassky, Minshew, & Just, 2006). Repetitive behaviour has been more difficult to study directly in neuroimaging experiments. Instead, fMRI studies have focused on cognitive functions that may underlie or contribute to repetitive behaviour such as change detection (Gomot et al., 2006), inhibitory control (Schmitz et al., 2006; Shafritz, Dichter, Baranek, & Belger, 2008) and set shifting (Schmitz et al., 2006; Shafritz et al., 2008). Aside from these core symptom domains, a number of fMRI studies have also examined other aspects of executive function such as verbal fluency (Gilbert, Bird, Brindley, Frith, & Burgess, 2008); planning (Just, Cherkassky, Keller, Kana, & Minshew, 2007) and spatial working memory (Luna et al., 2002; Silk et al., 2006), as well as visuospatial processing (Damarla et al., 2010), motor execution (Mostofsky et al., 2009) and reward processing (Schmitz et al., 2008). Overall, these studies have had a significant impact on the acceptance of ASD as neurobiological disorder, by providing evidence of multiple disrupted brain-behaviour relations in ASD. Furthermore, these studies demonstrated that complex behavioural symptoms could be broken down into recognizable neural components (Minshew & Keller, 2010). Taken together, fMRI studies of individual tasks suggest that ASD is a widely distributed disorder, involving multiple systems across the brain, and a possible decrease in cortical specialization in ASD, based on mild shifting of peak areas of cortical brain activity, across a variety of tasks (Anagnostou & Taylor, 2011). Thus, the neural underpinnings of ASD
cannot be found in only one network or system and are widespread, suggesting that integrative models are needed to understand this disorder.

1.5.2 Functional connectivity in ASD

The second phase of studies has focused largely on functional connectivity analyses in ASD. This body of work has formed the basis of the hypothesis that autism is a disorder of underconnectivity among brain regions, causing disruption in cortical networks (Minshew & Keller, 2010). Most of the evidence for this hypothesis comes from task-related functional connectivity fmRI (fc-fMRI) studies. In these studies, data from fMRI studies of individuals with ASD showed reduced correlations between blood-oxygen level dependent (BOLD) activation in cortical areas that activated at the same time. The first demonstration of this technique was by Just et al. (2004), in a language comprehension task. In this task, correlation of the time series of the activation between the various participating cortical areas was consistently lower for the ASD group than for the control participants (Just et al., 2004). This reduced correlation has subsequently been demonstrated across a wide range of tasks related to domains of ASD including face processing (Kleinhanhs et al., 2008; Koshino et al., 2008; Welchew et al., 2005), theory of mind (Kana, Keller, Cherkassky, Minshew, & Just, 2009), semantic reasoning (Sahyoun et al., 2010), language comprehension (Kana et al., 2006) and executive function proxies for repetitive behaviour such as inhibitory control (Kana, Keller, Minshew, & Just, 2007; Solomon et al., 2009), as well as working memory (Koshino et al., 2008; (Koshino et al., 2005), planning and problem solving (Just et al., 2007), motor function (Mostofsky et al., 2009; Villalobos, Mizuno, Dahl, Kemmotsu, & Müller, 2005) and an embedded figure task (Damarla et al., 2010). Long distance frontal-posterior connections may be commonly affected (Kana et al., 2009; Minshew & Keller, 2010). A small number of reports have found evidence of enhanced or increased functional connectivity. A recent report by Noonan, Haist, & Müller (2009) found that there were more extensive activation areas correlating with three cortical seed regions in the ASD group, suggesting that connectivity may be more diffuse. Two other studies found evidence of greater subcortical-cortical connectivity with the thalamus (Mizuno, Villalobos, Davies, Dahl, & Müller, 2006) and the caudate (Turner, Frost, Linsenbardt, McIlroy, & Müller, 2006). These studies all used a slightly different analysis approaches, involving the statistical removal of task-related effects from the BOLD signal time series, and performing inter-region correlations on the remaining residual signal. The study mentioned previously conducted by Sahyoun et al. (2010),
along with findings of decreased activation and underconnectivity in frontotemporal language areas, also showed evidence of increased activation and intact connectivity in occipito-parietal and ventral temporal networks, important for visuospatial skills and visual problem solving, which may provide evidence of a neural basis for enhanced visuospatial skills (Sahyoun et al., 2010). Overall, it may be that inefficient, not just decreased, connectivity is the problem in ASD, resulting from both decreased connectivity between many essential regions, along with possible overconnectivity between other, non-essential regions. This could result in increased noise and decreased signal in the system, reducing efficiency (Noonan et al., 2009). In addition, the results suggest that subcortical-cortical connectivity may be affected differently than cortical-cortical connectivity; the latter may in fact be hyperfunctional (Mizuno et al., 2006).

Functional connectivity has also been studied in non-task dependent paradigms. Recently, researchers have identified a network of areas of increased BOLD signal during rest, compared with the active part of a task, referred to as the “default-mode network”. It is now well accepted that this network includes midline structures such as medial prefrontal cortex, anterior and posterior cingulate, medial parietal cortex and precuneus, and medial temporal regions such as parahippocampal gyri (Greicius, Krasnow, Reiss, & Menon, 2003; Raichle et al., 2001). The default mode is of particular interest in ASD because it involves many of the same regions that are important for complex emotional and social processing, theory of mind and self-referential thought (Minshew & Keller, 2010). It is also known that connectivity within this network matures over development, with far less connectivity between these regions seen in children (Fair et al., 2008), making it a prime candidate for abnormalities due to growth dysregulation. Individuals with ASD may show reduced activity in the default mode in general (Kennedy, 2006), although this finding has not been reliably replicated and requires further investigation (Cherkassky et al., 2006; Kennedy & Courchesne, 2008). Furthermore, studying functional connectivity during resting state allows for investigation of long distance connections without any confounds from task performance or difficulty. Results of these recent studies support the hypothesis of reduced long distance connectivity between frontal and posterior regions (Cherkassky, Kana, Keller, & Just, 2006; Kana et al., 2006; Kennedy & Courchesne, 2008; Monk et al., 2009).
1.6 Rationale for the studies

As summarised above, further work is needed to determine more detailed measures of brain structure, and studies are lacking that examine the impact of age in a childhood sample. Structural and functional studies reviewed in previous sections all suggest that the neurological underpinnings of ASD are widespread throughout the brain, possibly due to an atypical maturational pattern that has particularly drastic effects on connectivity and functional networks. Further investigation of the pattern of development in ASD, rather than absolute differences at a particular age, is most critical to examine in depth, to improve our understanding of the disorder from a neurodevelopmental perspective. As such, studies should be conducted that focus on children across a range of ages and use age as the key variable of interest in their analyses. It will be helpful to use this approach across different structural and functional datasets, to provide converging evidence for atypical maturation as a mechanism of neurological etiology in ASD.

The three studies of this thesis apply this approach to cortical grey matter development, using measures of cortical volume, thickness and surface area; white matter development, using measures of diffusion; and functional development in areas important for set shifting, using functional magnetic resonance imaging (fMRI). Set shifting was chosen as a function of interest based on the executive function theory of ASD. In particular, evidence for the growth dysregulation hypothesis will be investigated in all three of these studies by examining the data for age by group interactions. The impact of growth dysregulation for underconnectivity in ASD will also be discussed.
2 Measures of cortical grey matter structure and development in children with autism spectrum disorder

2.1 Introduction

As reviewed in Section 1.2.1, abnormalities in brain volume are the most consistent neurobiological finding in ASD. Cortical grey matter can be analyzed more specifically in terms of cortical thickness and surface area, the two components of grey matter volume. As reviewed in Section 1.2.2, early investigations of cortical thickness generally showed increases in this measure in adults with ASD (e.g., Bailey et al., 1998; Hutsl er et al., 2007, Hyde et al., 2010) but some recent reports have been mixed (Chung et al., 2005; Hadjikhani et al., 2006, Wallace et al., 2010). The few studies of cortical thickness in children with ASD have tended to show mostly increases (Hardan et al., 2006). There is also some evidence that cortical thickness may decrease with age (Hardan et al., 2009) or at least that differences in cortical thickness between typically developing participants and those with ASD may change over development (Raznahan et al., 2010). Wallace et al. (2010) have suggested that the typical U-shaped maturational curve seen in grey matter cortical thickness (Shaw et al., 2008) may be atypically shifted leftward in ASD. Initial evidence from a small number of studies of cortical surface measures also suggests abnormalities in ASD (Hardan et al., 2004; Levitt et al., 2003; Nordahl et al., 2007; but see Raznahan et al., 2010 for an exception).

Although there have been a number of studies investigating brain volume in ASD, there are relatively few investigations on more detailed measures of grey matter. Investigation of differences in these measures of cortical grey matter may provide important indications of very early neuroanatomical developmental events in the ASD population. Furthermore, given that ASD is a developmental disorder and there is now a body of work documenting significant changes in grey matter indices over childhood (Shaw et al., 2008; Lenroot et al., 2009), it is important to investigate change in these measures in children across age. Investigation of the pattern of development in ASD, rather than absolute differences at a particular age, will provide

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greater understanding of the disorder from a neurodevelopmental perspective, and could be used to inform other critical diagnostic, genetic and treatment investigations of the disorder.

In the present study of children from 6 to 15 years of age, we investigated the relation between age and structural measures of volume, surface area and cortical thickness in ASD relative to a typically developing control group. I believe that this is the first report to examine cortical thickness in a larger group of only school-aged children with ASD using a vertex-by-vertex whole-brain approach. We hypothesized that children with ASD would show abnormalities in the typical maturation of cortex, evident in age by group interactions in measures of volume, surface area and cortical thickness, particularly in the frontal lobes, and that these abnormalities would be more pronounced at earlier ages.

2.2 Materials and Methods

2.2.1 Participants

Participants were 25 children, all male, diagnosed with an Autism Spectrum Disorder (ASD) (mean age = 10.9 years, range 6.8 – 15.4) and 63 male, typically developing (TD) control children (mean age = 11.3 years, range 6.5 – 15.8). Of the 25 ASD participants, 24 were recruited through the Autism Research Unit at the Hospital for Sick Children (Toronto, Canada), and were diagnosed by clinician experts supported by a research reliable Autism Diagnostic Observation Schedule (ADOS-G) (Lord et al., 2000) and Autism Diagnostic Interview-Revised (ADI-R) (Lord, Rutter, & Le Couteur, 1994). One ASD participant was recruited by parent interest in the study, and was not available for ADI/ADOS assessment, but had been given a diagnosis by a community clinician.

One of the ASD participants had a previous diagnosis of Generalized Anxiety Disorder, and two had a previous diagnosis of Attention Deficit Hyperactivity Disorder. Two ASD participants had an early childhood history of seizures, but had been deemed seizure free. One ASD participant was on medication (selective serotonin reuptake inhibitor, risperidol and methylphenidate). TD participants were screened and had no known history of psychiatric or neurological disorders.

Full scale IQ was measured using the 4-subtest Wechsler Abbreviated Scale of Intelligence (WASI, Wechsler, 2002) for 23 of the participants with ASD, and the majority (n=39) of the typically developing controls. One ASD participant was previously assessed with the Wechsler
Intelligence Scales for Children (WISC-IV, Wechsler, 2003), and one with the Leiter International Performance Scale Revised (Leiter-R, Roid & Miller, 1997). All ASD participants had IQ >70.

2.2.2 Imaging Parameters

High-resolution axial T1-weighted images were obtained for all of the children on a 1.5 Tesla GE scanner (FSPGR sequence, 116 slices; TR = 9 ms, TE=4.2 ms, flip angle = 15). Voxel size was 0.9375 x 0.9375 x 1.5 mm at 2 NEX.

2.2.3 Image Processing

T1 images were registered to the symmetric ICBM 152 template with a 12-parameter linear transformation (D. L. Collins, Neelin, Peters, & Evans, 1994), RF inhomogeneity corrected (Sled, Zijdenbos, & Evans, 1998), skull stripped (Smith, 2002) and tissue classified (Tohka, Zijdenbos, & Evans, 2004; Zijdenbos, Forghani, & Evans, 2002). Deformable models were then used to first fit the white matter surface for each hemisphere separately, followed by an expansion outward to find the grey matter/CSF intersection (Kim et al., 2005; MacDonald, Kabani, Avis, & Evans, 2000), resulting in 4 surfaces of 40,962 vertices each. From these surfaces the distance between the white and grey surfaces was used to measure cortical thickness (Lerch & Evans, 2005). The thickness data were blurred using a 20 mm surface-based diffusion blurring kernel (Chung, Robbins, Dalton, Davidson, Alexander, & Evans, 2005) and non-linearly aligned using surface based registration (Lyttelton, Boucher, Robbins, & Evans, 2007; Robbins, Evans, Collins, & Whitesides, 2004) prior to statistical analyses. Un-normalized, native-space thickness values were used in all analyses owing to the poor correlation between cortical thickness and brain volume (Ad-Dab'bagh et al., 2005; Sowell et al., 2007). Along with measures of cortical thickness at each of 81,924 vertices across the cortex, total volumes and surface area were estimated for the whole brain and each cortical lobe. This was accomplished by non-linearly warping each T1 image towards a segmented atlas (Chakravarty et al., 2009; Collins, Holmes, Peters, & Evans, 1995).

2.2.4 Statistical Analyses

Statistical analyses were performed on the brain volume, surface area and cortical thickness data. Effects of age and group alone were examined using analysis of variance, and differences in
these effects were examined using an analysis of covariance linear model. Here we allowed for separate age by imaging metric (thickness, surface area, etc.) slopes for the two groups (TD and ASD), and computed the marginal significance of the difference in intercepts and difference in slopes between the groups. In order for the intercept to be interpretable, we centred it at three different ages within the range of available data; at the younger end of the age range (7.5 years), in the middle (11 years) and at the older end of the age range (14.5 years). Results of vertex-based cortical thickness analyses were thresholded for statistical significance using the False Discovery Rate (FDR) correction at $q<0.05$, and at $q<0.10$ to indicate trends (Genovese, Lazar, & Nichols, 2002).

2.3 Results

2.3.1 Demographic Results

There was no significant difference in age between the two groups ($t(86)=-0.68$, $p=0.50$, n.s.). ASD participants had significantly lower full-scale IQ than the subset of typically developing children with IQ measures (ASD=104.52 vs. TD=114.95, $t(32.3)=-2.49, p=0.02$).

2.3.2 Imaging Results

2.3.2.1 Brain Volume

Differences between the ASD and TD groups in the relation between age and overall brain volume were evident in an age by group interaction in total brain volume (Figure 2.1) ($t(84) = -2.13, p = 0.036$). Inspection of this interaction revealed that brain volume was increased for the ASD group relative to the TD group at younger ages, but was significantly decreased relative to the TD group at older ages ($t(84) = -2.16, p = 0.033$).
Figure 2.1 Brain volume by age for the ASD and TD children. Shading represents the 95% confidence interval around the linear fits for each group. Pattern shows increased brain volume at younger ages for the ASD children compared to the TD children, and the opposite, decreased volume, at older ages.

For grey matter volume alone, the results were similar, with a trend toward an age by group interaction ($t(84) = -1.79, p = 0.076$), and a pattern of increased grey matter volume in ASD relative to the TD group at younger ages, and decreased grey matter at older ages (Figure 2.2). At the lobar level, the age by group interaction was also significant in occipital grey matter volume ($t(84) = -2.24, p = 0.03$) and approached significance in frontal grey matter volume ($t(84) = -1.85, p = 0.07$). Significant decreases with age were seen in the ASD group only in overall grey matter volume ($t(23) = -2.27, p = 0.025$), as well as frontal ($t(23) = -2.31, p = 0.023$), parietal ($t(23) = -2.061, p = 0.042$) and occipital lobe grey matter ($t(23) = -2.41, p = 0.018$).
Figure 2.2 Grey matter volume by age for the ASD and TD groups. Figure shows the same pattern of increased volume at younger ages for ASD children and decreased volume at older ages relative to control children as seen in overall brain volume.

2.3.2.2 Surface Area

Results of the age by group ANCOVA for surface area (Figure 2.3) showed the same pattern of results as in volume, with increased surface area at younger ages and decreased surface area at older ages. Although the age by group interaction did not reach statistical significance, there was a trend for overall surface area to decrease with age for the ASD group only (t(23) = -1.95, p = 0.054). In addition, at the lobar level an age by group interaction was seen in occipital lobe surface area (t(84) = -2.30, p = 0.023) and there was a trend toward significance in frontal surface area (t(84) = -1.78, p = 0.078). Surface area was significantly decreased in the ASD group.
relative to the TD group at the older end of the age range (centered at 14.5 years) in occipital lobes (t(84) = -2.31, p = 0.023).

Figure 2.3 Surface area by age for both the ASD and TD groups.
Figure shows the pattern of increased surface area at younger ages in ASD, and decreased surface area at older ages compared to controls, similar to the pattern seen in the overall brain volume and grey matter volume.

2.3.2.3 Cortical Thickness

The same pattern of results was seen in mean cortical thickness, with increased thickness at younger ages and decreased thickness at older ages in the ASD group compared to control children (Figure 2.4). Although the age by group interaction was not significant, mean cortical thickness decreased significantly with age in the ASD group only (t(23) = -2.37, p = 0.027). This pattern was also significant in the parietal lobes (t(23) = -2.76, p = 0.011) and approached significance in the frontal lobes (t(23) = -2.03, p = 0.054).
Figure 2.4 Mean cortical thickness across age in the ASD and TD groups.

Figure shows pattern of increased CT at younger ages in the children with ASD and decreased CT at older ages, compared to TD children.

The strongest effects seen in the regional analysis of cortical thickness were age effects, including thickening of the temporal pole, pre-central and superior temporal gyri and thinning of multiple other cortical areas in the TD group. In ASD children, similar results were seen. However, additional regions of decreased cortical thickness with age were also observed in the ASD group in bilateral medial parieto-occipital fissure/precuneus (LH: $t(23) = -4.75$, $p = 0.00009$, $q < 0.05$; RH: $t(23) = -4.06$, $p = 0.00049$, $q < 0.10$), medial cingulate/paracentral lobule (LH: $t(23) = -4.02$, $p = 0.0005$, $q < 0.05$; RH: $t(23) = -3.93$, $p = 0.0007$, $q < 0.10$) and left inferior frontal gyrus/BA 44 ($t(23) = -3.66$, $p = 0.001$, $q < 0.05$), that were not observed in the TD group. In two of these regions the cortex was significantly thicker for the ASD group relative to TD children at the younger end of the age range (centered at 7.5 years): the left medial parieto-
occipital fissure/precuneus \( t(84) = 4.88, p = 0.000005, q < 0.05 \) and left inferior frontal gyrus (BA44) \( t(84) = 4.25, p = 0.00005, q < 0.10 \) (Figure 2.5).

Figure 2.5 Areas of increased mean cortical thickness in the ASD group.

Cortical thickness was significantly increased for children with ASD compared to controls in BA 44 and medial precuneus at the younger end of the age range, but not at older ages.

To address any issues in matching, the results detailed above were verified in a smaller sample excluding the ASD participant without ADOS/ADI-R characterization and TD participants without measured IQ, and full-scale IQ was added as a covariate. Overall patterns remained the same, and most statistical results remained significant or were even improved. For example, the age by group interaction of grey matter went from a \( p \)-value of \( t(84) \ p = 0.076 \) to \( t(58) \ p = 0.092 \), whereas the local cortical thickness results in precuneus remained unchanged, from \( t(84) \ p = 0.00003 \) to \( t(58) \ p = 0.000003 \). The full sample employed in this study is thus very likely an
accurate reflection of the smaller, better-characterized cohort of subjects, and differences in IQ between the groups do not appear to account for the results found in this study.

2.4 Discussion

Overall, we found atypicalities in the relation between age and several structural measures of the cortex in ASD children. Evidence came from differing relations between the structural measures and age seen in brain volume, surface area and in cortical thickness for children with ASD compared to typically developing children. The results suggest a pattern of increased volume, surface area and cortical thickness at younger end of the age range of this sample (around 7.5 years), but decreased or similar volume, surface area and cortical thickness at the older end of this age range (around 14.5 years). The difference in developmental progression appeared most robust in overall brain volume (grey and white matter combined), where the age by group interaction was significant, in occipital and frontal lobes for overall grey matter and surface area. Although the age by group interaction was not significant in mean cortical thickness, significant age effects were seen in the ASD group only, and at the lobular level appeared stronger in parietal and frontal lobes. When examined using a regionally specific approach, two regions showed thicker cortex for the ASD group relative to the TD group at the younger end of the age range. Lastly, overall group differences were not seen when age was not considered in the model.

These findings strongly indicate that development is an important factor to consider in analyses of neural correlates of ASD and that cortical abnormalities associated with ASD change with age. The age range of this sample does not include pre-school children, where differences in brain volume are most often reported in the literature, suggesting that neuroanatomical effects persist past the pre-school period, at least until mid-childhood.

Brain volume results from the current study are generally consistent with the existing literature on this measure. The pattern of increased brain volume seen at the younger end of our age range is concordant with other studies that have found evidence of increased volume peaking in the preschool years. Although the majority of previous studies have suggested that differences in brain volume between ASD and TD individuals eventually normalize, there is debate about when this occurs, with some researchers finding continued increased volume in studies of older individuals with ASD. Our current results show a greater decrease in brain volume with age in the ASD group that eventually results in significantly decreased brain volume at the later ages of
our sample (mid-teenage years), a result not seen in the majority of previous volume analyses. However, the overall pattern of decrease with age is similar, and significantly decreased volume for an ASD group at older ages has been shown in at least one previous study (Courschesne et al., 2001). These discrepancies may be due to a number of factors, including the age range studied, and the characteristics of the ASD population. In this study we were looking at a tighter age range and specifically high-functioning individuals, relative to the extant literature.

Because of the small number of existing studies of surface area in ASD, an overall pattern has yet to emerge from the literature. Findings from the present study suggest that surface area may tend to decrease with age in children with ASD and that this may differ from typically developing children, contrary to some previous findings of a lack of relation between these measures (Raznahan et al., 2010). In our results, surface area is increased in ASD children at younger ages and decreased at older ages, relative to TD children, which is generally consistent with the findings of increased gyrification at younger ages, and decreasing gyrification with age in ASD (Hardan et al., 2004).

Cortical thickness has been reported to be increased in some studies of adults with ASD. Due to the small number of studies of cortical thickness conducted in ASD children only, a consensus has yet to emerge, but Hardan et al. (2006) found thicker cortex overall, as well as in temporal and parietal lobes, in a group of children with ASD aged 8-12 years, relative to an age-matched group of TD children using average measures of cortical thickness across lobes, as opposed to a vertex-based analysis. Here we did not see evidence of an overall increase in cortical thickness or in cortical thickness averaged across lobes for the whole group. However, we did find a pattern of increased cortical thickness in two regions of cortex, which were significantly increased at the younger end of the age range, and a relation between decreasing overall, parietal and frontal cortical thickness and age in the ASD group only. Since Hardan et al. (2006) did not use age as a covariate in their analyses it is not possible to know if they would have found any variation in lobar cortical thickness based on age. In addition, it is difficult to compare results from an averaging method to a vertex-based approach, which provides regionally specific detail.

When examining subjects from a much larger age range (10 – 65 years) Raznahan et al. (2010) found that at younger ages cortical thickness was decreased in ASD relative to typically developing controls, but increased relative to controls at older ages. They also reported that in
regions showing an age by group interaction, there was no relation between age and changes in cortical thickness in the ASD group, compared to a significant decrease in cortical thickness with age in the control group. Our results are in contradistinction to these findings; however, the age ranges of the two studies are quite different and the number of subjects overlapping with our age range was likely small, making it difficult to be sure of the sources of these discrepancies. In contrast, when examining change over time (on average 2 years), Hardan et al. (2009) found that there was evidence of a greater decrease in average cortical thickness in children with ASD relative to controls, in the whole brain, in the temporal and occipital lobes, and a trend in the frontal lobes, but significant in occipital cortex only. This overall pattern is consistent with the results observed in the present study, where decreases with age for overall and lobar cortical thickness were observed in the ASD group only, and where both regions emerging from the ANCOVA (left medial parieto-occipital fissure/precuneus and left IFG/BA44) showed steeper decreasing slopes with age in children with ASD across age compared to the typically developing children.

Our vertex-based analysis further showed that both groups showed similar regions of age-related changes in cortical thickness, except for the additional regions observed in the ASD group (the same regions that had group differences at the younger end of the age range). Therefore, our results suggest that there are indeed regions showing greater age-related cortical changes in ASD children 6 – 15 years of age, relative to typically developing children. Results may differ from the previous studies because of methods (vertex-based approach versus lobular averaging, allowing more detailed cortical analyses), age range of the sample and investigation of developmental change across this specific age range.

Of the two regions that emerged from the ANCOVA, the left IFG area has been implicated in previous structural studies of individuals with ASD. It has been suggested that the frontal lobes may be particularly affected by abnormal brain maturation processes in ASD (e.g., Carper et al., 2002), and abnormalities in the frontal lobes are one of the most consistently reported findings in the study of brain volume (Amaral et al., 2008). This hypothesis was also partially confirmed by the trends in the present study toward increased grey matter volume, surface area, and cortical thickness in the frontal lobes in the younger children with ASD. Furthermore, this region is known to be important both for language (Broca’s area) and executive function (Hill, 2004), two functional domains that are impaired in ASD (DSM-IV; American Psychiatric Association
Thus, the age by group findings appear to be particularly relevant to impairments in these areas, which can be seen even in high functioning children with ASD, as studied presently. These findings are consistent with the hypothesis that maturational abnormalities in these focal areas may underlie deficits in these domains.

The second area, the precuneus, has recently been functionally linked to a central role in self-referential thought and self-processing, including functions such as first person perspective taking, episodic memory retrieval, and the experience of agency (see Cavanna & Trimble, 2006 for a review of the role of the precuneus in these functions). In addition, the precuneus has been identified along with other midline structures as part of the “default mode” of brain function (Raichle et al., 2001). Therefore it has been proposed that the precuneus may be an essential neural correlate of self-consciousness, engaged in self-related mental representation (Cavanna & Trimble, 2006). A number of recent studies have shown that self-referential thought and its neural correlates may be abnormal in ASD (Lombardo, Barnes, Wheelwright, & Baron-Cohen, 2007; Lombardo et al., 2010) and that this may be critically important to difficulties in ASD with theory of mind (Lombardo & Baron-Cohen, 2011). Structurally, the precuneus is unique because of the strategic position that it occupies in the structural hierarchy of the cerebral cortex (Bullmore & Sporns, 2009). Parts of the medial posterior cortex, including the precuneus and posterior cingulate, are identified as putative hub regions due to their unusual anatomical properties, including dense connectivity, short average path length to other regions and the participation in a large proportion of short paths between other regions, both in anatomical (Hagmann et al., 2008; Iturria-Medina, Sotero, Canales-Rodriguez, Aleman-Gomez, & Melie-Garcia, 2008) and in functional brain networks (Hagmann et al., 2008; Achard, Salvador, Whitcher, Suckling, & Bullmore, 2006). We have shown that developmental changes in brain complexity are most marked in the precuneus region (Misic, Mills, Taylor, & McIntosh, 2010). Atypical development of this area in ASD may have far-reaching effects and relate to the poorer development of functional connectivity in ASD, as summarized in Section 1.3.2 above, and discussed further in Chapter 5 (General Discussion).

2.5 Limitations

The analyses conducted in this study were cross-sectional, and maturational change with age is best examined by a longitudinal study with a large cohort, where changes with age can be
measured within participants. However, these studies are time and resource intensive. Many initial investigations of possible age-related phenomena are cross-sectional. The results from the current study, suggest that a large-scale longitudinal study would be warranted, and the next step to verify and expand these current findings.

2.6 Conclusions

Overall, these findings lend support to the growth dysregulation hypothesis (Akshoomoff et al., 2002) by providing further evidence that maturation of the cortex is atypical in children with ASD. Our data demonstrate that these changes are found in volume, and its contributing components surface area and cortical thickness, and evolve over childhood. As mentioned earlier, cortical thickness and surface area are thought to depend on different maturational sources. It has been hypothesized that cortical thickness is related to dendritic arborisation and pruning within grey matter (Huttenlocher, 1990) or changes in myelination at the interface of grey and white matter (Sowell et al., 2004), whereas surface area is dependent on division of progenitor cells in the periventricular area during embryogenesis (Chenn & Walsh, 2002) and varies with degree of cortical folding/gyrification. Therefore, these results indicate that neural abnormalities in ASD are likely the result of dysregulation of more than one neurodevelopmental process, affecting neuronal growth, pruning, myelination and cell division, and leading to widespread effects in the brain. These widespread effects have particularly significant consequences for connectivity and the development of functional networks, and may explain the pervasive atypicalities in these areas seen in ASD.
3 White matter and development in children with an autism spectrum disorder

3.1 Introduction

As summarized in Section 1.2.3, DTI is a magnetic resonance imaging technique that measures the diffusion of water within tissue (Basser, Matiello & LeBihan, 1994a, Basser, Matiello, & LeBihan, 1994b, Basser, Matiello & LeBihan, 1996). Measures of DTI include longitudinal diffusivity (Dmax), radial diffusivity (Drad), mean diffusivity (MD) and the composite measure of fractional anisotropy (FA), all of which reflect various aspects of white matter structure including axonal integrity, myelin integrity and degree of anisotropic diffusion (see Section 1.2.3). Thus, several measures can be extracted from DTI neuroimaging studies, which will contribute to a fuller understanding of possible differences in white matter development in ASD.

FA has been the most common measure reported in previous studies of DTI in ASD (see Section 1.2.3). Although locations have been variable (orbitofrontal, medial prefrontal, temporal lobe, corpus callosum, arcuate and uncinate fasciculi, cerebellar tracts, internal capsule), and different techniques have been used, including tractography and voxel-based techniques, the majority of studies have found evidence of reduced FA in children (Barnea-Goraly et al., 2010; Barnea-Goraly et al., 2004; Brito et al., 2009; Cheung et al., 2009; Ke et al., 2009; Sundaram et al., 2008; Sahyoun et al., 2009) and adults with ASD (Catani et al., 2008; Keller et al., 2007; Lee et al., 2007; Thakkar et al., 2008; Pardini et al., 2009), which has typically been interpreted as evidence for decreased organization and coherence within white matter pathways. However, at least a few recent studies have found evidence of regions of increased FA in ASD, in samples of young children (Ben Bashat et al., 2007) and adolescents (Cheng et al., 2010). Despite the variability in brain areas reported, the large number of groups finding evidence of abnormalities in white matter measures itself suggests that there is credence to these findings. However, because FA is an aggregate and non-specific measure, investigation of the individual eigenvalues are needed to better interpret any differences in DTI that may exist between clinical and control groups. Recent studies have begun to report the sources of these differences in radial and axial
A recent report showed increases in mean diffusivity in the arcuate fasciculus in adolescents with ASD, mainly due to increases in radial diffusivity, implicating the myelin component of white matter in ASD pathophysiology (Fletcher et al., 2010) and another reporting increases in children with ASD in radial diffusivity in the whole brain, the corpus callosum and internal capsule (Shukla, Keehn, Lincoln, & Müller, 2010). However, at least one study has highlighted widespread decreases in FA within frontal parietal and temporal lobes, driven by decreases in axial but not radial diffusivity (Barnea-Goraly et al., 2010). A consensus has yet to emerge on the significance of abnormalities in axial versus radial diffusivity in this population.

The aim of this study was to further investigate the growth dysregulation hypothesis using detailed measures of diffusivity, in a group of children with ASD and a group of typically developing (TD) children. It was hypothesized that there would be an atypical maturation pattern in the children with autism, evident in age by group interactions, as well as differences between the groups, within defined white matter tracts in measures of diffusion. We also wanted to better characterize the sources of any observed interactions or group differences by examining longitudinal, radial and mean diffusivity, along with FA.

3.2 Methods

3.2.1 Participants

Participants were 34 children diagnosed with an Autism Spectrum Disorder, recruited through the Autism Research Unit at the Hospital for Sick Children (Toronto, Canada), and diagnosed by clinician experts supported by a research reliable Autism Diagnostic Observation Schedule (ADOS-G) (Lord et al., 2000) and Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994). One of the ASD participants had a previous diagnosis of Generalized Anxiety Disorder, one had a previous diagnosis of a learning disability, and two had a previous diagnosis of Attention Deficit Hyperactivity Disorder. TD participants were screened and had no known history of psychiatric or neurological disorders.

Datasets were visually inspected to ensure data quality. Datasets were excluded if they contained too much motion (evident in inter-volume or inter-slice misalignments or severe signal drop outs), or did not have full brain coverage, which resulted in the exclusion of the 13 datasets for the ASD group, for a total sample of 23 (4 female, mean age = 11.07 years, range 6-14). Twenty-
three age- and gender-matched typically developing control children were then chosen from a larger pool of DTI scans (4 female, mean age = 11.13 years, range 6-14). The average absolute discrepancy in age after matching was 4.84 months (SD=5.25 months). For children with ASD, diagnosis was established by expert clinical judgement using DSM-IV criteria and by ASD cutoff scores on Autism Diagnostic Observation Schedule (ADOS-G, Lord et al., 2000) and Autism Diagnostic Interview-Revised (ADI-R, Lord et al., 1994). A measure of IQ (WASI, Wechsler, 2002) was collected on all of the participants with ASD, and all but one of the typically developing controls. Relative to this group of 22 controls, ASD participants had significantly lower full-scale IQ (ASD=100.57, range = 58 – 138, SD = 19.75) vs. TD=117.64, range = 90 – 141, SD = 10.79), t(43)= -3.574, p=0.001). Demographic data is summarized in Table 3.1. Parents of all participants provided informed consent, and the study was approved by the Research Ethics Board of SickKids Hospital.

Table 3.1 Summary of demographic characteristics of the two groups.

<table>
<thead>
<tr>
<th></th>
<th>TD</th>
<th>ASD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (females)</td>
<td>23 (4)</td>
<td>23 (4)</td>
</tr>
<tr>
<td>Mean Age (SD)</td>
<td>11.13 (2.27)</td>
<td>11.07 (2.34)</td>
</tr>
<tr>
<td>Mean IQ (SD)</td>
<td>117.64 (10.79)</td>
<td>100.57 (19.75)</td>
</tr>
</tbody>
</table>

### 3.2.2 Imaging Parameters

Subjects were scanned on a 1.5 T magnetic resonance imaging (MRI) scanner (GE Signa Excite, Waukesha, USA) with an 8 channel array head coil. Diffusion tensor images were acquired using a single-shot spin echo planar imaging sequence. Diffusion gradients were twice refocused to reduce the effect of eddy currents (Reese, Heid, Weisskoff, & Wedeen, 2003). Thirty-five non-collinear directions were employed with a b-value of 1000 s/mm2. Three non-diffusion weighted volumes were acquired. Slices were 3 mm thick and prescribed to sufficiently cover the cerebrum (approx. 40 slices) and were oriented parallel to the anterior commissure–posterior commissure (AC-PC) axis of the subject. In-plane resolution was 2.5 mm × 2.5 mm.

### 3.2.3 Image Processing

Image processing was performed using a combination of FSL (Saunders, Vickers, Zhang, De Stefano, Brady, and Matthews, 2004), AFNI (Cox, 1996) and Camino (Cook et al., 2006) software packages. All volumes were registered to one of the non-diffusion weighted volumes using an affine transformation (FLIRT, Smith et al., 2004) to correct for motion and residual
eddy current effects. To correct for signal-loss in slices affected by motion or pulsation artefacts, the diffusion tensor was estimated from the corrected data using the RESTORE method (Chang, Jones, & Pierpaoli, 2005). From the estimated diffusion tensor, fractional anisotropy (FA), mean diffusivity (MD), longitudinal (Dmax = first eigenvalue) and radial diffusivity (Drad = average of second and third eigenvalues) were calculated. To perform a group analysis, all FA maps were aligned to the JHU-ICBM DTI-81 FA template (included in FSL) using non-linear registration (FNIRT, Andersson, Jenkinson and Smith, 2007). The same warp was applied to MD, Dmax and Drad maps. Data were blurred by a 3 mm full-width half-max Gaussian kernel. In each subject, average values for FA, Dmax, Drad, and MD were computed for major fibre tracts in the brain, as defined in the JHU-ICBM DTI-81 white matter atlas. A list of these tracts is presented in Table 3.2, and ROIs are shown on average FA and MD for all participants in Figure 3.1.

Table 3.2 Tracts from the JHU-ICBM DTI-81 template used in the analysis.

<table>
<thead>
<tr>
<th>Tracts included in analysis</th>
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<tbody>
<tr>
<td>Genu of corpus callosum</td>
</tr>
<tr>
<td>Body of corpus callosum</td>
</tr>
<tr>
<td>Splenium of corpus callosum</td>
</tr>
<tr>
<td>Fornix (column and body of fornix)</td>
</tr>
<tr>
<td>Cerebral peduncle</td>
</tr>
<tr>
<td>Anterior limb internal capsule</td>
</tr>
<tr>
<td>Posterior limb internal capsule</td>
</tr>
<tr>
<td>Retrolenticular internal capsule</td>
</tr>
<tr>
<td>Anterior corona radiata</td>
</tr>
<tr>
<td>Superior corona radiata</td>
</tr>
<tr>
<td>Posterior corona radiata</td>
</tr>
</tbody>
</table>
3.2.4 Statistical Analysis

In this cross-sectional analysis, effects of age, group and age by group interaction were examined using a general linear model, testing for effects of group (with age covaried), age (for each group), and group-by-age interactions within these tracts. Results corrected for multiple comparisons using the False Discovery Rate (FDR; Genovese et al., 2002) method are also presented.

3.3 Results

3.3.1 Group differences

When age was covaried and effects of group were examined, there were a few regions showing significant group differences (Table 3.3). In almost all cases, effects showed increased measures for the ASD group relative to the TD group. The ASD group had greater Dmax, Drad, and
Table 3.3 Regions showing group differences between the ASD and TD groups for FA, longitudinal, radial and mean diffusivity, with age covaried.

Results for non-lateralized structures (e.g., corpus callosum) are indicated under the "left" heading. (*= p<0.05, **=p<0.01, Degrees of freedom = (1, 42)). All results are for increased measures for the ASD group, except for the right uncinate fasciculus, which was in the opposite direction (TD>ASD).

<table>
<thead>
<tr>
<th>FA</th>
<th>Dmax</th>
<th>Drad</th>
<th>MD</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
</tr>
<tr>
<td>Post thalamic/optic radiation</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Retrolicular internal capsule</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Superior corona radiata</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Posterior corona radiata</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sagittal stratum</td>
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<td>-</td>
<td>4.73</td>
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<tr>
<td>Fornix/stria terminalis</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Superior fronto-occipital fasciculus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Uncinate fasciculus</td>
<td>-</td>
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</tbody>
</table>
MD in posterior thalamic radiation/optic radiation and the uncinate fasciculus, as well as increased Dmax and MD in left sagittal stratum. Dmax alone was increased for the ASD group in the retrolenticular part of the internal capsule, and Drad was increased for the ASD group in left posterior corona radiata. No group differences were observed in FA. The only region showing decreased diffusivity for the ASD group was in Dmax in right uncinate fasciculus. After applying the Bonferroni correction for multiple comparisons, no group differences remained significant.

### 3.3.2 Age effects

Cross-sectional age effects were examined in the typically developing group. As expected, multiple regions throughout the brain were seen to mature with age, with Drad and MD being the most sensitive to age effects (Table 3.4). In all regions reported, FA increased with age, while Dmax, Drad, and MD decreased with age, consistent with many reports in the literature and with the neuroanatomical age-related increases in white matter density over childhood (e.g., Lebel et al., 2008; Snook et al., 2005). Many of these results remained significant following correction for multiple comparisons (see Table 3.4).

A similar analysis of age in the ASD group showed far fewer regions with a significant relation with age (Table 3.5). Results for only two regions (superior longitudinal fasciculus, and cingulum) remained significant following correction for multiple comparisons. This was explored more fully by examining age by group interactions.

### 3.3.3 Age by group interactions

Age by group interactions were evident in a number of regions including frontal, posterior, interhemispheric and long-distance tracts. These interactions, consistent with our hypothesis, indicated significant differences in the relation between age and diffusion measures for children with ASD relative to typically-developing children (Table 3.6). Such interactions were evident in frontal and posterior tracts, as well as the corpus callosum, and occurred in longitudinal, radial and mean diffusivity rather than FA.
Table 3.4 Areas showing significant change with age in the typically developing group for FA, longitudinal diffusivity (Dmax), radial diffusivity (Drad), and mean diffusivity (MD).

Results for non-lateralized structures (e.g., corpus callosum) are indicated under the “left” heading. (*= p<0.05, **=p<0.01, †=significant after FDR correction for multiple comparisons, Degrees of freedom = (1, 42)). In all regions, FA increased with age, and longitudinal, radial, and mean diffusivity decreased with age.

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>Dmax</th>
<th>Drad</th>
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<td>Right</td>
</tr>
<tr>
<td>Anterior corona radiata</td>
<td>12.70</td>
<td>0.002**†</td>
<td>9.53</td>
<td>0.006**†</td>
</tr>
<tr>
<td>Superior corona radiata</td>
<td>12.69</td>
<td>0.002**†</td>
<td>12.97</td>
<td>0.002**†</td>
</tr>
<tr>
<td>Posterior corona radiata</td>
<td>29.77</td>
<td>&lt;0.001**†</td>
<td>22.52</td>
<td>&lt;0.001**†</td>
</tr>
<tr>
<td>Anterior limb internal</td>
<td>11.16</td>
<td>0.003**</td>
<td>4.57</td>
<td>0.045*</td>
</tr>
<tr>
<td>capsule</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior limb internal</td>
<td>-</td>
<td>-</td>
<td>4.68</td>
<td>0.042*</td>
</tr>
<tr>
<td>capsule</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrolenticular internal</td>
<td>-</td>
<td>-</td>
<td>6.48</td>
<td>0.019*</td>
</tr>
<tr>
<td>capsule</td>
<td></td>
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<td>8.84</td>
<td>0.007**†</td>
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</tr>
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<td>Sup. longitudinal fasciculus</td>
<td>5.94</td>
<td>0.024*</td>
<td>9.36</td>
<td>0.006**</td>
</tr>
<tr>
<td>Cingulum</td>
<td>6.62</td>
<td>0.018*</td>
<td></td>
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</tr>
<tr>
<td>Uncinate fasciculus</td>
<td>7.73</td>
<td>0.011*</td>
<td></td>
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</tr>
<tr>
<td>Cerebral peduncle</td>
<td>-</td>
<td>-</td>
<td></td>
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</tr>
<tr>
<td>Post. thalamic/optic rad</td>
<td>14.14</td>
<td>&lt;0.001**†</td>
<td>6.60</td>
<td>0.018†</td>
</tr>
<tr>
<td>Sagittal stratum</td>
<td>6.43</td>
<td>0.019*</td>
<td></td>
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</tr>
<tr>
<td>External capsule</td>
<td>14.37</td>
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<td>7.46</td>
<td>0.012*</td>
</tr>
<tr>
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<td></td>
</tr>
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<td>Fornix/stria terminalis</td>
<td>8.18</td>
<td>0.009**†</td>
<td></td>
<td></td>
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<tr>
<td>Sup. fronto-occipital fasciculus</td>
<td>-</td>
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</table>
Table 3.5 Areas showing significant change with age in the ASD group for FA, longitudinal diffusivity (Dmax), radial diffusivity (Drad), and mean diffusivity (MD).
Results for non-lateralized structures (e.g., corpus callosum) are indicated under the "left" heading. (*= p<0.05, **=p<0.01, †=significant after FDR correction for multiple comparisons, Degrees of freedom = (1, 42)). In all regions, FA increased with age, and longitudinal, radial, and mean diffusivity decreased with age.

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>Dmax</th>
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<tr>
<td></td>
<td>Left</td>
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<tr>
<td>Superior corona radiata</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Posterior corona radiata</td>
<td>10.91</td>
<td>0.003**</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Anterior limb internal capsule</td>
<td>4.96</td>
<td>0.037*</td>
<td>-</td>
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</tr>
<tr>
<td>Posterior limb internal capsule</td>
<td>5.40</td>
<td>0.03*</td>
<td>-</td>
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</tr>
<tr>
<td>Retrotricular internal capsule</td>
<td>6.88</td>
<td>0.016*</td>
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<td>-</td>
</tr>
<tr>
<td>Superior longitudinal fasciculus</td>
<td>4.35</td>
<td>0.049*</td>
<td>5.93</td>
<td>0.024*</td>
</tr>
<tr>
<td>Cingulum</td>
<td>4.72</td>
<td>0.041*</td>
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<tr>
<td>Uncinate fasciculus</td>
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<tr>
<td>Cerebral peduncle</td>
<td>7.73</td>
<td>0.011*</td>
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<td>Post. thalamic/optic radiation</td>
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<td>Superior longitudinal fasciculus</td>
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<td>Posterior thalamic/optic radiation</td>
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Table 3.6 Regions showing significant age by group interactions for diffusion measures FA, longitudinal diffusivity (Dmax), radial diffusivity (Drad), and mean diffusivity (MD).
Results for non-lateralized structures (e.g., corpus callosum) are indicated under the "left" heading. (*= p<0.05, **=p<0.01, †= significant after FDR correction for multiple comparisons, Degrees of freedom = (1, 42)). In all regions, diffusion measures decreased with age for the TD group, with little or no change with age for the ASD group.

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>Dmax</th>
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<tr>
<td>Superior corona radiata</td>
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<tr>
<td>Anterior corona radiata</td>
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<tr>
<td>Anterior limb internal capsule</td>
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<tr>
<td>Corpus callosum (body)</td>
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<td>Corpus callosum (splenium)</td>
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</table>
Inspection of these interactions showed that these effects were due to significant decreases with age in these DTI metrics in the TD group, relative to little or no change with age in the ASD group. Interactions were seen in the body, genu and splenium of the corpus callosum, bilateral anterior corona radiata, right anterior and posterior limbs of the internal capsule, bilateral superior longitudinal fasciculus and the left posterior thalamic radiation (including optic radiation). All but one of these regions showed interactions in the longitudinal diffusivity (Dmax) measure, with the majority also showing these effects in Drad and MD. Results for the left anterior corona radiata, the genu of the corpus callosum and the left posterior thalamic radiation/optic radiation, which showed significant interaction effects across all measures except FA, are shown in Figures 3.2 – 3.10. Graphs of all other significant interaction effects can be found in Appendix 1. After correction for multiple comparisons, results remained significant for longitudinal diffusivity in the genu of the corpus callosum only.

![Left Anterior Corona Radiata](image)

Figure 3.2 Longitudinal diffusivity in the left anterior corona radiata for ASD and TD groups, plotted by age.
Figure 3.3 Radial diffusivity in the left anterior corona radiata for ASD and TD groups, plotted by age.

Figure 3.4 Mean diffusivity in the left anterior corona radiata for ASD and TD groups, plotted by age.
Figure 3.5 Longitudinal diffusivity in the genu of the corpus callosum for ASD and TD groups, plotted by age.

Figure 3.6 Radial diffusivity in the genu of the corpus callosum in ASD and TD groups, plotted by age.
Corpus Callosum (Genu)

Figure 3.7 Mean diffusivity in the genu of the corpus callosum for ASD and TD groups, plotted by age.

Left Posterior Thalamic Radiation/Optic Radiation

Figure 3.8 Longitudinal diffusivity in the left posterior thalamic radiation/optic radiation for ASD and TD groups, plotted by age.
Figure 3.9 Radial diffusivity in the left posterior thalamic radiation/optic radiation for ASD and TD groups, plotted by age.

Figure 3.10 Mean diffusivity in the left posterior thalamic radiation/optic radiation for ASD and TD groups, plotted by age.
3.4 Discussion

These data provide evidence of atypical maturational patterns in white matter tracts in the brain in children with ASD compared to typically developing children. While absolute group differences were relatively subtle, we found evidence of significant age by group interactions in a number of white matter tracts, including the corpus callosum, posterior tracts and regions in the frontal lobes. Inspection of these age by group interactions showed that in all cases, measures of diffusivity were decreasing, as expected, with age in the typically developing group, but changing very little in the ASD group. Although this is cross-sectional data, this strongly suggests atypicalities in the maturational pattern in the ASD group on diffusion measures, consistent with the hypothesis that brain growth is dysregulated in ASD. Most of these effects came not in FA, but in longitudinal, radial and (consequently) mean diffusivity. It has been previously suggested (Cheng et al., 2010) that the period of late childhood/early adolescence represented in our sample may be a time at which directionality of FA differences may be switching (i.e., greater FA before this time period, and decreased FA afterward), which may explain why age by group and group difference findings were lacking in the measure of FA in our results. In addition, since FA reflects relative disparity between Dmax and Drad, if both are changing this will result in a lack of change in FA. Indeed, examination of more specific measures of diffusivity (Dmax, Drad) were more appropriate for this age range and revealed age-related atypicalities in this data set in the children with ASD – specifically, the little or no age-related decreases in measures of diffusivity, whereas the typically developing children showed the expected decreases in diffusivity with age.

Regions showing these age by group interactions included frontal white matter tracts such as anterior corona radiata and the anterior limb of the internal capsule. Frontal lobe differences have been reported in previous DTI studies (e.g., Sundaram et al., 2008, Cheng et al., 2010), as well as other structural studies suggesting that the frontal lobes are particularly affected by volumetric abnormalities early in life (e.g., Carper and Courchesne, 2005, Herbert et al., 2004). In addition, the frontal lobes have been implicated from a functional perspective in cognitive theories of autism symptomatology, such as impairments in executive function (e.g., Hill, 2004), social processing (Castelli et al., 2002) and language (Just et al., 2004).
However, posterior tracts, responsible for carrying sensory information, were also involved, with the same interaction pattern found in the posterior limb of the internal capsule and the posterior thalamic radiation/optic radiation. Abnormalities in internal capsule diffusivity were also reported by Shukla et al. (2010), who highlighted the possible link between such abnormalities and motor and sensory symptoms often reported in ASD, and pointed out that the impact of sensorimotor abnormalities on subsequent development in ASD remains to be understood. Previous studies have reported findings in sensory areas of the brain in structural studies of ASD. For example, reduced occipital white matter volume has been reported in ASD (Bonilha et al., 2008) and Hyde et al. (2010) found group differences between adults with and without ASD in cortical thickness in areas of primary auditory and visual cortex, as well as perceptual association areas. The latter study provided some of the first evidence that atypicalities in brain regions involved in sensory processing could also be involved in ASD, consistent with the atypical sensory processing frequently reported in ASD.

Finally, age by group interactions were also seen in all areas of the corpus callosum (body, genu and splenium), the major connecting pathway between the cerebral hemispheres. The genu was the only region where interaction results were still significant after correction for multiple comparisons. The corpus callosum has been previously implicated in DTI and MRI studies of ASD (e.g., Alexander et al., 2007, Frazier and Hardan, 2009, Shukla et al., 2010), and our present data provide further evidence that white matter microstructure of the corpus callosum is affected in ASD. The finding that the genu showed the most reliable of these interaction effects may be of significance, considering other studies that have suggested particular involvement of the frontal lobes in the neural basis of ASD (e.g., Carper et al., 2002; Carper & Courchesne, 2005; Courchesne and Pierce, 2005). Furthermore, if the major inter-hemispheric tract connecting the frontal lobes is maturing abnormally in ASD, this would certainly impact the communication between the frontal lobes and contribute to the problems of long-range under-connectivity reported in this population (Sections 1.2.3 and 1.5.2).

3.5 Limitations

Group effects, some age effects in both groups, and all but one of the interaction results were not significant after correction for multiple comparisons. This is likely a result of reduced statistical power due to the small sample size; a large number of ASD datasets had to be excluded due to
movement artefacts. Also, abnormalities in the cerebellar white matter were not investigated in this study, due to insufficient slice coverage. In addition, there was a significant difference in IQ between the groups; it is possible that differences between them could have been affected by this variable. Finally, the analyses conducted in this study were cross-sectional, and sample size was small when stratified by age. Maturational change with age is best examined by a longitudinal study with a large cohort, where changes with age can be measured within participants, rather than extrapolated across different participants. However, these studies are time and research intensive. Many initial investigations of possible age-related phenomena are cross-sectional. The results from the current study, suggest that a large-scale longitudinal study would be warranted, and the next step to verify and expand these current findings.

3.6 Conclusions

Overall, our results are consistent with the hypothesis that white matter structure is affected in ASD, and also that the growth and development of the white matter tracts with age may be atypical in this population, in that they did not show the typical decreases with age seen in the typically developing group. We found that specific measures of diffusion (Dmax and Drad), rather than the aggregate measure of FA, were more sensitive in this age range. These more specific measures should, with an increased number of studies in the future, contribute greater understanding to the pathophysiology of the developmental white matter abnormalities in ASD.
4 Age-related atypicalities in the fMRI response to set shifting in children with an autism spectrum disorder

4.1 Introduction

As summarized in Section 1.4.3, it is hypothesized that symptoms of ASD may be related to impairment in executive function, an umbrella term referring to cognitive functions important for goal-directed activity such as planning, working memory, inhibition and cognitive flexibility, as well as the initiation and monitoring of action. It is well established that cognitive abilities such as executive function mature with age, as the brain develops. The prefrontal cortex, known to be important in executive function, also has a protracted maturation relative to other parts of the neocortex; the frontal lobes continue to develop until the third decade of life (Huttenlocher & Dabholkar, 1997; Yakolev & Lecours, 1967). Critically, findings from structural and functional neuroimaging suggest that the trajectory of cortical development and organization is abnormal in children with ASD. Overall, a “growth dysregulation hypothesis” of neuropathology of ASD has emerged, characterized by overgrowth early in life, followed by abnormally slowed or even arrested growth in some regions (Akshoomoff et al., 2002). Abnormal neuroanatomical development would undoubtedly have profound effects on the development of cognitive functions in this population.

Investigations of the relation between the function of brain regions important for executive function and age in typical and clinical populations are critical to the understanding of what is going awry under conditions of abnormal neural development. The neural correlates of cognitive flexibility have been investigated primarily in samples of healthy adults, with the Wisconsin Card Sorting Task, intradimensional (ID) extradimensional (ED) tasks, and a variety of other “task switching” paradigms. In these task switching paradigms, participants are presented with bidimensional stimuli, which contain attributes relevant to both tasks. They need to switch back and forth between the stimuli during the task, attending to the relevant attribute, based on cues or sequence instruction. In this body of literature, prefrontal regions are the most commonly implicated areas for successful task performance (Dove, Pollmann, Schubert, Wiggins, & von Cramon, 2000; Hampshire & Owen, 2006; Konishi et al., 1998; Konishi et al., 1999; MacDonald, Cohen, Stenger, & Carter, 2000; Monchi, Petrides, Petre, & Dagher, 2001; Rogers, Andrews, Grasby, Brooks, & Robbins, 2000; Sohn, Ursu, Anderson, Stenger, & Carter, 2000;
Zanolie, Leijenhorst, Rombouts, & Crone, 2008), along with parietal cortex (Asari, Konishi, Jimura, & Miyashita, 2005; Bunge, Kahn, Wallis, Miller, & Wagner, 2003; Kimberg, Aguirre, & D'Esposito, 2000; Hampshire & Owen, 2006; Sohn et al., 2000), and basal ganglia (Nagano-Saito et al., 2008; Monchi et al., 2001; Sohn et al., 2000).

There are only two studies examining the neural correlates of cognitive flexibility in children, and both have compared differences in activation for adults and children. One study found increasing recruitment of prefrontal and parietal regions in adults as compared to children, with both groups showing robust caudate activation for shifts of attention between dimensions of colour and shape (Casey et al., 2004). The second study found that adults showed increased activation in inferior prefrontal cortex, parietal lobe and putamen on a task that involved switching attention between the horizontal (left/right) and vertical (top/bottom) components of the spatial location of a coloured dot (Rubia et al., 2006). In addition, whole-brain regression analyses with age across all subjects showed progressive positive age-related changes for this task in right inferior prefrontal cortex, insula, precentral gyrus, superior temporal lobe as well as right orbitofrontal gyrus, left mesial frontal cortex, right and left caudate and putamen, and left tail of the caudate. Negative correlations with age were observed in right dorsolateral prefrontal cortex, left insula, mesial frontal gyrus, left superior and middle temporal lobes, right thalamus and hippocampus, left occipital cortex, and bilateral cerebellum (Rubia et al., 2006). From these findings, the authors suggest that task-specific frontostriatal and frontal cortical networks for cognitive control functions mature progressively from childhood to mid-adulthood. It appears that in some cases maturation results in increasing activation, but in other cases decreases in activation, depending on the area and the protocol used.

Two studies have also investigated the neural correlates of set shifting in adults with ASD. On a spatial switch task identical to that used by Rubia et al. (2006), despite a lack of behavioural performance differences between the groups, ASD individuals showed significantly increased brain activation relative to control participants in left middle frontal gyrus, inferior parietal lobe, insula, caudate, and putamen, as well as right fusiform, inferior temporal, and cingulate gyri (Schmitz et al., 2006). The authors suggested that the adults with ASD used a similar network as control adults, but greater activation of task-relevant areas was required, possibly due to abnormal brain development leading to inefficient neural network recruitment (Schmitz et al., 2006).
In a second study (Shafritz et al., 2008), participants were shown a series of shapes with infrequent target shapes (squares, circles and triangles of various sizes and colours). They were asked to push one button for non-target shapes (the majority of trials) and another button for any exemplars of the target shape. Target shapes switched every two runs between circles, and triangles, resulting in “maintain” runs (runs where the target did not change) and “shift” runs (where the target was changed). The average activation toward target shapes could therefore be compared between “maintain” and “shift” runs. Again behavioural performance was comparable between the groups, but compared with control subjects, participants with autism showed reduced activation for “target shift” events in frontal, striatal and parietal regions. In addition, within the autism group, the severity of restricted, repetitive behaviours was negatively correlated with activation in anterior cingulate and posterior parietal regions (Shafritz et al., 2008).

Overall, the literature shows that frontal, striatal and parietal areas and networks are clearly implicated in tasks of cognitive flexibility, and there is some initial evidence for abnormalities in these areas in adults with ASD. However, further investigation is needed specifically in children with ASD, and taking age and brain maturation into account. The present study investigated the relation between neural activity and age in a functional neuroimaging task of set shifting, in a group of children with ASD and a group of typically developing children. We hypothesized that there would be an atypical relation between activity related to shifting and age in the children with ASD, compared to the typically developing group, evident in age by group interactions in brain regions known to be important for set shifting, such as the right prefrontal cortex. One hypothesis is that activation in these areas may increase with age in the typically developing children, reflecting increased recruitment; and this pattern may be altered in the ASD group, showing little or no age-related increases. In addition, behavioural measures of set shifting and repetitive behaviour were collected, to provide more detailed information about set shifting behaviour in this particular sample, and possible relation to measures of repetitive symptoms of ASD. To our knowledge, there have been no studies of neural correlates of cognitive flexibility in children with ASD, nor any investigating the relation between neuroimaging findings and age in this population.
4.2 Methods

4.2.1 Participants

26 children diagnosed with an Autism Spectrum Disorder were recruited through the Autism Research Unit at the Hospital for Sick Children (Toronto, Canada), and diagnosed by clinician experts supported by a research reliable Autism Diagnostic Observation Schedule (ADOS-G) (Lord et al., 2000) and Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994). Participants were excluded if they were on any medication at the time of the study (1 participant). In addition, 7 children were unable to successfully perform the task inside the scanner, despite pre-training, and the experiment was aborted (note: all children successfully completed at least the structural scan, and no children had to leave the scanner abruptly for behavioural reasons). After preprocessing, datasets were excluded if they contained too much motion, which resulted in the exclusion of 4 additional datasets for the ASD group, for a total sample of 14 (2 female, mean age = 10.81 years, range = 7-13). All of the children in this remaining group were considered to be high-functioning. 14 age-matched datasets were then selected from a larger group of typically developing children who completed the task (3 female, mean age = 10.42 years, range = 7-13). All but one of the age-matched pairs were also gender-matched. The average absolute discrepancy in age after matching was 4.93 months (range = 0.4 – 14.36 months).

4.2.2 Behavioural Testing

Participants were administered a measure of IQ (WASI, Wechsler, 2002), except for one participant from the ASD group who had already received a recent assessment of IQ using the Weschler Intelligence Scale for Children (WISC-IV). Participants also completed the following selected neuropsychological measures of cognitive flexibility: the Animal Sorting and Inhibition subtests from the NEPSY-II (Korkman, Kirk & Kemp, 2007), the Opposite Worlds subtest from the Test of Everyday Attention for Children (TEA-Ch, Manly, Robertson, Anderson & Nimmo-Smith, 1999) and Trails A & B of the Trail Making Test (TMT). In the NEPSY-II Animal Sorting task, children are given a set of different cards with pictures of animals in different landscape settings. The pictures are designed to provide multiple ways of sorting the cards into groups. The children are instructed to arrange the cards into 2 groups of 4 cards in as many ways as they can within a given time period. Scores are obtained based on the number of correct sorts,
and errors including number of repeated sorts and number of non-rule-based sorts. In the NEPSY-II Inhibition task, children see a page of black and white squares and circles, and up and down arrows, which they need to name out loud. In the final, third condition of this task (Switch), they must pay attention to the colour of the stimulus in question. If the stimulus is white, they say the correct name, and if the stimulus is black they must say the opposite name (e.g., “square” for circle, “up” for a downward-pointing arrow, thus switching back and forth between the two rules depending on colour. In the TEA-Ch Opposite Worlds task, children must respond aloud to a sequence of number “1”s and “2”s arranged in a trail across the page. In the first part of the task (Same World) they read the numbers as written, as quickly as they can without making mistakes. In the second part of the task (Opposite World) they have to inhibit the correct labelling of the numbers, and instead say “2” aloud when they see a 1, and say “1” when they see a 2. Scores are obtained for the time in seconds to complete two trials each of the Same and Opposite Worlds, administered in the following order: Same, Opposite, Opposite, Same, requiring the child to switch between the two rules and thus is also considered by the test authors to be a measure of switching as noted in the manual (Manly et al., 1999). Trails B of the TMT is also a task that involves switching, children must draw a line connecting alternating numbers and letters in ascending order (e.g., 1-A, 2-B, etc). Scores on these neuropsychological measures were unavailable for three children, one from the TD group and two from the ASD group. As well, scores for the NEPSY-II Inhibition subtest were not collected on one additional participant from the ASD group.

In addition, parents of participants completed the Behaviour Rating Inventory of Executive Function (BRIEF, Gioia, Isquith, Guy, & Kenworthy, 2000), a parent report measure of difficulties with everyday executive function. Scores on the BRIEF were unavailable for one child from the TD group. Parents of the children with ASD also completed the Repetitive Behaviour Scale – Revised (RBS-R, Bodfish, Symons, Parker, & Lewis, 2000) provides detailed information on repetitive behaviour by surveying behaviour from 6 different categories: Stereotyped (apparently purposeless movements or actions that are repeated in a similar manner), Self-Injurious (movement or actions that have the potential to cause redness, bruising, or other injury to the body, and that are repeated in a similar manner), Compulsive (behaviour that is repeated and is performed according to a rule, or involves things being done “just so”), Ritualistic (performing activities of daily living in a similar manner), Sameness (resistance to
change, insisting that things stay the same) and Restricted (limited range of focus, interest or activity) (Bodfish et al., 2000). Scores on the RBS-R were unavailable for two of the children from the ASD group. Parents of all participants provided informed consent, and the children provided informed assent; the study was approved by the Research Ethics Board of SickKids Hospital.

4.2.3 Experimental Task

Participants received a detailed explanation of the task before entering the scanner and practiced the protocol until they demonstrated that they understood the task. Each trial required a 2-alternative forced choice between compound stimuli of two dimensions (Dimension 1: clown fish, Dimension 2: aquatic plant). These stimuli were designed to be child-friendly, to facilitate testing with both TD and ASD children. Example stimuli are shown in Figure 4.1.

![Example stimuli](image_url)

Figure 4.1 Examples of set shifting task stimuli.

The session comprised 3 fMRI runs of 10 sets each. To begin each run, participants were shown a series of all possible targets, each lasting 1 second, with a string of “X”s above to represent the text that would later appear to indicate the target. This was followed by a fixation cross for 20 seconds. Then, they were shown an explicit instructional cue for 1 second, which showed them a picture of the target stimulus, a particular exemplar from one of the dimensions (e.g., blue fish). A trial stimulus then appeared, with two of the compound stimuli, one on the right of the screen and one on the left. The task was to indicate the location of the picture that included the target stimulus. The response was made by pressing a button with either their right or left index finger, to indicate either the right or left side of the screen. The relevant dimension of the compound stimulus (fish or plants) appeared in the foreground of all stimuli for that set of trials. Each trial lasted 3 seconds, with a blank screen buffer if the child responded before the 3 seconds had elapsed. A non-response was considered incorrect. After it was established that the child was responding consistently to the cue (counterbalanced between 3 or 4 consecutive correct
responses), a shift occurred. The shift was either intradimensional (within dimension 1: e.g., from blue fish target to yellow fish target) or extradimensional (shift from dimension 1 to 2: from fish target to plant target, or vice versa). After each trial, the child received feedback on his or her response. Each time a shift occurred the participant was shown a new cue, which gave them the new instruction to follow. Participants received “gold coins” for each correct response (shown on the correct feedback screen) and periodically (6 times per run) saw an update on the number of coins in their “piggy bank”. The progression of a set is depicted in Figure 4.2 below (feedback and update trials are not shown for simplicity).

Figure 4.2 Progression of a set within the experimental task.

While inside the scanner, visual images were projected to the participants using LCD goggles, and responses given via two keypads, placed under the right and left index fingers respectively and connected to a computer running Presentation software (Neurobehavioural Systems, Berkley, CA).

4.2.4 Imaging Parameters

Children were scanned on a 1.5 T MRI scanner (GE Signa Excite, Waukesha, USA) with an 8 channel array head coil. Anatomical scans were acquired using the 3D FSPGR sequence, producing volumes of T1-weighted axial slices, with voxels = 0.9375 mm x 0.9375 mm, slice thickness of 1.5 mm, TR = 9 ms. Functional data were acquired using the 2D Spiral In/Out sequence, producing volumes of T2*-weighted axial slices, with voxels 3.75 mm x 3.75 mm, with a slice thickness of 5 mm, TR = 2 s.
4.2.5 Image Processing

Structural images were skull stripped and registered to a standard Talairach space. The first three volumes of functional data were discarded to allow for stabilization of magnetic field. Functional images were corrected for motion and slice time offset, aligned to the individual’s structural image, spatially smoothed at 10 mm (FWHM) Gaussian kernel, and scaled to percent signal change.

4.2.6 Statistical Analysis

BOLD signal change data were analysed using Analysis of Functional Neuroimages (AFNI) software (Cox, 1996) in an event-related protocol, based on the time at which the cognitive shift occurred. This was operationalized as the time at which the cue to shift (ID cue or ED cue) was presented, for successful shifts only. A successful shift was defined as one that was following by 3 consecutive correct responses, without making any errors. Sets where this criterion was not achieved were excluded from the analysis. Data were fit to a General Linear Model (GLM) using the following explanatory variables: example targets, ID cues, ED cues, individual trials, feedback and bank updates. Data were then modelled using a gamma variate function, generating individual beta maps for the activation of ED cues versus fixation baseline, ID cues versus fixation baseline, and an ED-ID contrast. For the group analysis, an ANCOVA of age and group was conducted on the averaged Beta coefficients from the GLM for ED cues and the ED-ID contrast.

In addition, average signal change on clusters emerging from the age by group analysis were correlated with behavioural scores on neuropsychological measures of switching for both groups of children, and with scores of repetitive behaviour on the RBS-R, ADOS and ADI-R for the ASD group.

4.3 Results

4.3.1 Behavioural Results

4.3.1.1 IQ

There was no significant difference between the two groups for full-scale IQ (TD = 116.64 vs. ASD = 113.21, t(26) = 0.587, p = 0.56).
4.3.1.2 Cognitive Flexibility

ASD participants had significantly elevated scores (indicative of greater impairment) on all individual subscales and three composite measures of everyday executive function as rated by their parents (BRIEF, Goia et al., 2000). This included the Shifting subscale (t(25)=4.66, p<0.001) which measures difficulties with changes in routine and other rigid or inflexible behaviour. Sample items from this subscale include, “Resists changes in food, routine, places, etc.”, and “Thinks too much about the same topic”.

On the Animal Sorting task from the NEPSY-II, ASD children had significantly lower scaled score performance than the TD group for both total number of correct sorts (t(23)=2.91, p=0.008) and when errors were included in the scaled score (t(23)=2.82, p=0.010). There was also a trend for ASD children to achieve lower z-scored reaction times to complete Trails B of the TMT (t(23) = 1.76, p=0.091). On all other neuropsychological measures of switching, there was no significant difference in performance between the two groups.

Children with ASD also demonstrated some overall slowing of responses, with significantly slower z-scored reaction times on Trails A of the TMT (connecting numbers only) (t(23) = 2.07, p=0.05), as well as time to name stimuli on the NEPSY-II Inhibition subtest (t(22)=2.20, p=0.039).

4.3.1.3 Repetitive Behaviour

Information about repetitive behaviour in this sample was available from both the ADOS and the ADI-R. The average score on the Repetitive/Restricted behaviour section of the ADOS algorithm for this group was 3.71 (SD=1.68) with no child obtaining a score of 0. Developmentally, as assessed by the ADI-R, the average score on Repetitive/Restricted behaviour was 7.29 (SD=2.13), with no child obtaining a score below the diagnostic cut-off level of 3.

More detailed information on repetitive behaviour was gathered from the RBS-R Questionnaire, completed by parents. Proportion of items endorsed for each of the six categories and overall score are shown in Figure 4.3. These data show that the most strongly endorsed subscale was Ritualistic Behaviour, followed by Sameness and Restricted Behaviour. Items from the Self-Injurious Behaviour subscale were not highly endorsed in this sample.
4.3.1.4 Task Performance

The ASD and TD groups performed similarly with respect to errors and reaction times on the fMRI task in the scanner. Firstly, there were no significant differences between the TD and ASD groups on the number of sets removed for errors, either in the ED (t(26)=0.585, p=0.563) or ID conditions (t(26)=0.106, p=0.916). However, there was a significant overall effect of shift type, with significantly more sets removed for errors in the ED condition (ED = 4.713 vs. ID = 3.18, F(1, 26) = 15.17, p=0.001), and no shift type by group interaction (F(1, 26) = 0.402, p = 0.532). This indicates that the extradimensional shifts were more difficult than intradimensional shifts, as would be expected, and that this effect was seen equally in both groups. There was no overall effect of shift type (ED vs. ID) on reaction times to the first trial after the shift (F(1, 26) = 0.582, p=0.452), and similarly no shift type by group interaction for this measure (F(1, 26) = 0.096, p=0.759), again indicating similar behavioural performance for the two groups. The task was designed to have similar behavioural performance (i.e., be easy enough that the children with ASD could complete the task), thus we could analyse the imaging results without being confounded by performance. This was part of the original study design, and similarity in behavioural performance has been seen in other studies (e.g. Schmitz et al., 2006, and Shafritz et al. 2008, as reviewed above).
4.3.1.5 Imaging Results

4.3.1.5.1 Group Results

4.3.1.5.1.1 Extradimensional Shifts versus Fixation Baseline

Group differences were observed in 6 brain regions, at a p-value threshold of 0.01, uncorrected, and clusters of at least 12 voxels in size. Coordinates of peak activation are presented below in Table 4.1 and shown pictorially in Figure 4.4. In all of these regions, activation was greater for the ASD group compared to the TD group.

Table 4.1 Regions where group differences were observed for activation to ED shifts versus fixation baseline (p<0.01, uncorrected; clusters=12 voxels or greater).

<table>
<thead>
<tr>
<th>Region</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>L inf parietal/BA 40</td>
<td>-42</td>
<td>-50</td>
<td>56</td>
</tr>
<tr>
<td>R middle temporal gyrus</td>
<td>68</td>
<td>-34</td>
<td>-10</td>
</tr>
<tr>
<td>R precentral gyrus</td>
<td>38</td>
<td>-16</td>
<td>60</td>
</tr>
<tr>
<td>L middle temporal gyrus</td>
<td>-64</td>
<td>-54</td>
<td>0</td>
</tr>
<tr>
<td>L middle frontal gyrus/BA 10</td>
<td>-38</td>
<td>44</td>
<td>16</td>
</tr>
<tr>
<td>L precuneus</td>
<td>-30</td>
<td>-64</td>
<td>46</td>
</tr>
</tbody>
</table>

Figure 4.4 Regions where group differences were observed for activation to ED shifts versus fixation baseline. In all cases, activation was greater for the ASD group as compared to the TD group.

4.3.1.5.1.2 Extradimensional Shifts versus Intradimensional Shifts

Group differences were observed in 3 brain regions, at a p-value threshold of 0.01, uncorrected, and clusters of at least 12 voxels in size, in the contrast between extradimensional and intradimensional shifts. Co-ordinates of peak difference in activation are presented below in Table 4.2 and shown pictorially in Figure 4.5. In all of these regions, the difference for the ASD group was greater than for the TD group.
Table 4.2 Regions where group differences were observed for difference in activation to ED shifts versus ID shifts (p<0.01, uncorrected, clusters=12 voxels or greater).

<table>
<thead>
<tr>
<th>Region</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>R postcentral gyrus</td>
<td>26</td>
<td>-34</td>
<td>66</td>
</tr>
<tr>
<td>R inferior parietal</td>
<td>34</td>
<td>-58</td>
<td>40</td>
</tr>
<tr>
<td>L middle frontal gyrus/BA 10</td>
<td>-46</td>
<td>48</td>
<td>16</td>
</tr>
</tbody>
</table>

Figure 4.5 Regions where group differences were observed for difference in activation to ED shifts versus ID shifts. In all cases, difference in activation was greater for the ASD than the TD group.

4.3.1.5.2 Age by Group Interactions

4.3.1.5.2.1 Extradimensional shifts versus Fixation Baseline

Age by group interactions were observed in five cortical regions, at a p-value threshold of 0.01, uncorrected, and clusters of at least 12 voxels in size. Coordinates of peak activation are presented below in Table 4.3 and clusters shown in Figure 4.6. In all of these regions, signal change to extradimensional cues versus baseline was seen to decrease with age in the ASD group, but increase or show little change with age in the TD group. Graphs of average signal change for each cluster versus age, showing the age by group interactions, are shown in Figure 4.7.

Table 4.3 Regions where age by group interactions were observed for activation to ED shifts versus fixation baseline (p<0.01, uncorrected, clusters = 12 voxels or greater)

<table>
<thead>
<tr>
<th>Region</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>R inf parietal/BA 40</td>
<td>68</td>
<td>-20</td>
<td>20</td>
</tr>
<tr>
<td>L inf occipital gyrus</td>
<td>-30</td>
<td>-84</td>
<td>-14</td>
</tr>
<tr>
<td>R cuneus</td>
<td>18</td>
<td>-92</td>
<td>26</td>
</tr>
<tr>
<td>R insula</td>
<td>44</td>
<td>-8</td>
<td>0</td>
</tr>
</tbody>
</table>
L cingulate gyrus (near caudate)

Figure 4.6 Regions showing age by group interactions in BOLD signal change to ED shifts versus fixation baseline (p<0.01, uncorrected, cluster size 12 voxels or greater).
These effects were due to decreasing activation with age for the ASD group, but increases or no age-related change in the TD group.
An age by group interaction was observed in only one cortical region, at a p-value threshold of 0.01, uncorrected, and clusters of at least 12 voxels in size. Coordinates of peak activation difference are presented below in Table 4.4 and the cluster shown in Figure 4.8. In right middle frontal gyrus/BA 10, the difference in average signal change between the ED and ID shifts increased with age in the ASD group, whereas this difference showed little or no change with age in the TD group (Figure 4.9), remaining around the zero mark.
Table 4.4 Regions where age by group interactions were observed for difference in activation to ED shifts versus ID shifts (p=0.01, uncorrected, cluster=12 voxels or greater) for the extradimensional vs. intradimensional shifts.

<table>
<thead>
<tr>
<th>Region</th>
<th>x</th>
<th>y</th>
<th>z</th>
</tr>
</thead>
<tbody>
<tr>
<td>R middle frontal gyrus/BA 10</td>
<td>49</td>
<td>55</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 4.8 Right middle frontal gyrus/BA 10, showing age by group interaction in BOLD signal change to ED shifts versus ID shifts (p<0.01, uncorrected, cluster size = 12 voxels or greater).

Figure 4.9 Age by group interaction in right middle frontal gyrus/BA 10.
4.3.1.5.3 **Behavioural correlations**

Signal change in regions showing age by group interactions were correlated with behavioural scores. Only a few significant correlations were observed. For regions showing age by group interactions in BOLD signal change to ED shifts versus fixation baseline, as reported above, significant correlations were observed within the entire sample between average signal change in the right insula cluster and scaled scores on the Animal Sorting subtest of the NEPSY (Total Correct SS, $r=0.56$, $p=0.004$; Combined SS, $r=0.049$, $p=0.013$). For the ASD group, average signal change in the right insula cluster ($r=0.57$, $p=0.033$), and the right cuneus cluster ($r=0.71$, $p=0.004$), also correlated with the score on the Communication subscale of the ADOS.

For regions showing age by group interactions in BOLD signal change to ED shifts versus ID shifts, as reported above, a significant correlation was observed within the entire sample between average signal change in the right middle frontal gyrus/BA 10 cluster and the number of ED sets completed ($r=0.41$, $p=0.029$). This correlation also held in the ASD group alone ($r=0.65$, $p=0.011$).

4.4 **Discussion**

Based on the behavioural results, there was evidence of executive function impairment in the ASD group, both in everyday situations (from the BRIEF), and on some, but not all, of the neuropsychological measures. The group showed evidence of cognitive flexibility impairments in particular on the Animal Sorting subtest of the NEPSY-II, and, to a lesser extent the TMT (Part B). Scores on the Animal Sorting task were also related to functional results in one of the significant brain regions, the right insula. The ASD group also showed evidence of repetitive and restricted behaviour on the ADOS, ADI-R and the RBS-R. However, correlations between neuropsychological measures of flexibility and behavioural measures of restricted/repetitive behaviour were not observed.

Behavioural performance on the fMRI task revealed that ED shifts were more difficult, as more sets were removed for errors in the ED condition. This provides some verification that the experimental manipulation was successful. However, this pattern of increased errors did not differ between the groups, indicating that the groups were matched on behavioural performance. Thus, differences seen in brain activation are not due to differences in performance on the task.
Group differences were seen in brain activation to ED shifts, in a number of cortical regions. Greater activation for the ASD group to ED shifts (versus fixation baseline) was seen in left parietal cortex/BA 40, bilateral middle temporal gyrus, right precentral gyrus, left middle frontal gyrus/BA 10, and left precuneus, and to ED shifts (versus ID shifts) in right postcentral gyrus, right inferior parietal cortex and left middle frontal gyrus/BA 10. The fact that greater activation was seen for the ASD group in all cases suggests that, despite matched behavioural performance on the task, the ASD group may have needed to recruit more activity in these regions, many known to be important for cognitive flexibility, in order to complete the task successfully. Thus, this relatively easy task of cognitive flexibility was behaviourally within the abilities of the children with ASD, but still required greater neural resources to complete than the TD age-matched children.

Furthermore, age by group interactions revealed differences between the groups in the relation between brain activation to ED shifts and age. In total, six cortical regions showed significantly different patterns of activation with age for the ASD and TD groups. In the typically developing group, increases in activation to ED shifts (versus baseline) with age were seen in right inferior parietal (BA 40), left inferior occipital gyrus, right cuneus, right insula and left cingulate, whereas the ASD group showed decreases or no change in activation with age in these regions. In the ASD group, activation to ED shifts (versus ID shifts) showed an increase with age in right middle frontal gyrus/BA 10, but no change was seen in the TD group. These findings could reflect either reduced recruitment of these areas with age compared to the typically developing group, or the recruitment of alternative regions and networks, since performance on the task did not differ between groups.

Of these regions, a number have been previously implicated in neuroimaging studies of cognitive flexibility. The differences seen in right and left middle frontal gyrus/BA 10 are consistent with a large body of previous work showing the importance of the prefrontal cortex in executive function in general and cognitive flexibility in particular. This evidence comes from lesion and neuropsychological studies, as well as primate research, and many neuroimaging studies. In particular, patients who have sustained lesions to the prefrontal cortex have difficulty on neuropsychological measures of flexibility such as the WCST (Stuss & Alexander, 2007). Seminal work by Dias, Robbins & Roberts (1996) showed that damage to DLPFC of monkeys caused a specific impairment in shifting set, on the ID-ED task previously described. A host of
neuroimaging studies have confirmed the importance of prefrontal cortex in cognitive flexibility, though specific regions have differed (e.g., Konishi et al., 1998; Konishi et al., 1999, Monchi et al., 2001, Zanolie et al., 2008; Hampshire & Owen, 2006, Rogers et al., 2000; MacDonald et al., 2000; Dove et al., 2000; Sohn et al., 2000). The fact that frontal activation was particularly anticipated in this task, but showed age-related increase only in the ASD group, may be due to task ease. The inclusion of ‘hint’ stimuli, telling the children to switch, likely made the task very easy for the typical children, such that they did not need to recruit as much frontal lobe function for task performance as the children with ASD. In contrast, the children with ASD showed an increase in frontal activation with age, perhaps reflecting increasing use of frontal areas for this task, as their set-switching skills may improve with age, even with a simple task.

The parietal lobe has also been implicated in cognitive flexibility in a growing number of neuroimaging studies (e.g., Asari et al., 2005; Kimberg et al., 2000; Sohn et al., 2000; Bunge et al., 2003; Hampshire & Owen, 2006; Schmitz et al., 2006; Shafritz et al., 2008). Some studies have found bilateral activations; others seem to show a bias toward activation in the left hemisphere (e.g., Asari et al., 2005; Kimberg et al., 2000; Sohn et al., 2000). It is hypothesized that parietal cortex is important for maintaining relevant rule associations over a delay (Bunge et al., 2003). In a previous study, regions in right inferior and left mesial parietal cortices showed increased activation, bilaterally, for adults with ASD compared to control adults on a switching task (Schmitz et al., 2006), but in another adult study, an area in left intraparietal sulcus (IPS) showed reduced activation on target detection tasks which required a response shift (Shafritz et al., 2008). Thus, there is strong evidence for involvement of parietal lobes in cognitive flexibility functions, and initial evidence for atypicalities in function of parietal cortex in ASD, but variability in the location and laterality of parietal areas involved. These discrepancies could be due in part to the age range studied and the protocol differences that may preferentially engage left or right hemisphere processing.

Executive function in general has been widely reported to involve cingulate activity, but primarily in the anterior portion (Carter, Botvinick & Cohen, 1999; Botvinick, Carter & Cohen, 2004), with a main role in conflict monitoring and detection. Activation of the anterior cingulate cortex has also been shown specifically for studies of cognitive flexibility (e.g., Shafritz et al., 2005). It is hypothesized that the anterior cingulate cortex is important in set shifting tasks for monitoring errors associated with response competition (Carter et al., 1999). However, the
cluster found in this study was more posterior, and bordered on the caudate. It is more likely that this cluster reflects basal ganglia activation, which, as previously summarized, is commonly seen in tasks of cognitive flexibility as well (Nagano-Saito et al., 2008; Monchi et al., 2001; Sohn et al., 2000; Casey et al., 2004; Rubia et al., 2006) and this region has already been implicated in the two investigations of cognitive flexibility in adults with ASD (Schmitz et al., 2006; Shafritz et al., 2008).

Although the insula has been less frequently identified in reviews or meta-analyses of executive function, some studies have shown that the insula (specifically the right anterior insula) is functionally connected to other regions important for executive function, and may also play a role in monitoring of task performance (e.g., Eckert et al., 2009). In addition, at least one recent study of cognitive flexibility also showed activation in the insula in a task switch condition (Dove et al., 2000). In this study, a region in cuneus/precuneus was also more active in the task switch condition, which is also consistent with the current findings of an age by group interaction in this area. The precuneus has been implicated in at least one other study of task switching, and the authors suggested the precuneus activity may increase with the attentional demands for detection of stimulus attributes, and may be particularly important for shifting attention (though not exclusively) (Barber & Carter, 2005). The fact that we found that children with ASD showed decreasing activation with age, whereas typically developing children showed mainly increasing activations, is consistent with the latter group improving their ability to monitor and switch during task performance, moving towards adult levels. It is confirmatory that the children’s behavioural performance on the animal sorting task (which assesses mental flexibility and set-switching) also correlated with the BOLD signal in this brain area.

4.5 Limitations

The main limitation of this study is that the fMRI results were no longer significant after correction for multiple comparisons. This is likely a result of reduced statistical power due to the small sample size across this age range, particularly when scientifically the data need to be analysed stratified by age. However, the findings still provide interesting indications of alterations in functional patterns in the ASD group as compared to the typically developing children, and these findings will inform data-driven hypotheses for future studies. Also, activation in the cerebellum was not investigated in this study, due to insufficient slice coverage;
the whole cerebellum could only be included in a portion of the children (those with smaller heads). In addition, functional connectivity was not investigated in this analysis, and this might have given additional insight into group and developmental differences in the ASD group on this measure. However, techniques for the linking of structural and functional measures are still being refined, but will be pursued with these datasets in the future. Lastly, maturational change with age is best examined by a longitudinal study with a large cohort, where changes with age can be measured within participants, rather than extrapolated across different participants. However, these studies are time and research intensive, and many initial investigations of possible age-related phenomena are cross-sectional. The results from the current study, suggest that larger-scale longitudinal studies of important cognitive functions known to be impaired in ASD could be useful, and the next step is to verify and expand the current findings.

4.6 Conclusions

Overall, the findings of this study provide evidence that activation in areas known to be important for cognitive flexibility is different for children with ASD. This lends weight to the hypothesis that executive functions may indeed be atypical in ASD, as hypothesized by many investigators (e.g., Hill, 2004). Furthermore, the relation between BOLD activation to ED shifts (versus baseline, and versus ID shifts) and age in a number of critical functional regions is different for children with ASD compared to typically developing children. Together, these results may provide insight into the neural basis of impairments in the domain of cognitive flexibility seen in individuals with ASD. The finding of atypicalities in age-related change for the ASD group is also consistent with other evidence showing atypical maturational patterns in structural brain development. Along with the consistent evidence of atypical growth in brain volume, results from the studies in Chapters 2 and 3 strongly support the idea that brain anatomy develops according to an atypical trajectory in ASD. Anatomical differences, and associated connectivity differences could explain the differences in functional maturation seen in this study.
5 General Discussion

The studies in this thesis explored differences in the developmental patterns of brain structure and function between children with ASD and typically developing children. I pursued this question with three converging approaches: measures of cortical grey matter, measures of white matter in the brain and measures of brain activation during a task that taps a function challenging for children with ASD. These three studies allow me to present a multifaceted picture of atypical brain maturation across both structural and functional measures in children with ASD, that advances our understanding of the impact of underlying neuroanatomical differences on brain function and ultimately the behaviours characterising children with ASD, as summarised and discussed below.

The results from the structural gray matter study (Chapter 2) provide further evidence that maturation of the cortex is atypical in children with ASD. Our data demonstrate that these changes are found in volume, surface area and cortical thickness, and evolve over childhood. The results suggest a pattern of increased volume, surface area and cortical thickness at younger end of the age range of this sample (around 7.5 years) compared to typically developing children, but decreased or similar volume, surface area and cortical thickness at the older end of this age range (around 14.5 years). This pattern appeared across all three measures, but was most robust in overall brain volume (grey and white matter combined). These results are consistent with the large body of evidence that has emerged showing early brain overgrowth in young children with ASD. However, the pattern of age-related changes in brain volume over childhood has not been reliably established. The data from the current study demonstrate that the overgrowth is evident until mid-childhood; followed by a steady decrease – an atypical developmental pattern – such that overall volumes are reduced compared to controls in the teenage years. When cortical thickness was examined using a regionally specific approach, two regions, known to be important for functions of language (left IFG/Broca’s area) and social cognition (medial precuneus), showed thicker cortex for the ASD group relative to the TD group at the younger end of the age range. These findings are also consistent with the early overgrowth model, but the demonstration of regionally restricted effects is very interesting for our understanding of more specific brain-behaviour relations: that these effects are more marked in brain regions implicated in functions that are deficit in children with ASD. Lastly, overall group differences were not
seen for children with ASD relative to typically developing controls in these structural measures when age was not considered in the model. This highlights that it is the pattern of development, rather than absolute differences at a particular age, that is abnormal and most critical to examine in depth; the failure to examine data in light of ASD as a neurodevelopmental disorder would result in missing tremendous information and insights.

Results from the DTI study (Chapter 3) are consistent with previous evidence that white matter structure is affected in ASD, and also that the growth and development of white matter tracts may be atypical in this population. We found age by group interactions in a number of white matter tracts throughout the brain, including the corpus callosum, regions in frontal lobes and posterior tracts. Inspection of these interactions showed that in all cases, measures of diffusivity demonstrated decreasing diffusion, consistent with increasing structural constraints, with age in the typically developing group, but changing very little in the ASD group. Although this study was cross-sectional, these data strongly suggest atypicalities in the maturational pattern in the ASD group on diffusion measures, reinforcing the hypothesis that brain growth is dysregulated in ASD, and illustrating that this dysregulation includes white as well as grey matter. Most of these effects came not in the composite measure of FA, but in longitudinal, radial and (consequently) mean diffusivity. This specificity may help us understand further the pathophysiology of the white matter abnormalities reported in previous studies (Section 1.1.2). Regions showing atypical relations with age were seen in frontal white matter projection fibres such as anterior corona radiata and the anterior limb of the internal capsule, which project from frontal cortex to subcortical structures of the pons, and medial and anterior nuclei of the thalamus. Posterior tracts in the posterior limb of the internal capsule were also affected, which contains afferent corticospinal and sensory fibres, as was the posterior thalamic radiation/optic radiation, which are important pathways linking the thalamus with occipital and parietal lobes. Finally, atypical age relations were also seen in all areas of the corpus callosum (body, genu and splenium), the major connecting pathway between the cerebral hemispheres. These data suggest that white matter atypicalities in ASD are found in tracts throughout the brain, both anterior and posterior projection fibres, and also in major interhemispheric tracts. As the affected anterior fibre tracts were largely major, long-range pathways that connect cortical and subcortical regions, this aligns with the long-range underconnectivity reported in ASD (Cherkassky et al., 2006; Kennedy & Courchesne, 2008; Monk et al., 2009). These long-range fibre pathways play a
critical role in communication among brain regions, allowing the integration of information crucial to complex cognitive and social functions. The findings of the implication of posterior tracts associated with sensory regions may be linked with the frequently reported sensory abnormalities (hyper- or hypo-acuities, intense attention to stimulus details, etc.) seen in these children. These results are also compatible with the understanding of the neurological correlates of ASD being widespread throughout the brain (Anagnostou & Taylor, 2011) and provides further evidence from DTI of white matter abnormalities in ASD (see Section 1.1.2) and structural support for the functional underconnectivity model of ASD (Section 1.3.2.).

Findings of the set-shifting fMRI study (Chapter 4) present evidence that, in a group of children with ASD who showed evidence of executive impairment, the relation between BOLD activation to ED shifts (versus baseline, and versus ID shifts) and age is different for children with ASD compared to typically developing children. These differences occurred in six cortical regions, including a number that have been previously implicated in neuroimaging studies of cognitive flexibility, such as middle frontal gyrus in prefrontal cortex, parietal lobe, cingulate, insula and precuneus. These age-related group differences most frequently showed as increases with age in the BOLD signal in the typically developing children, but decreases for the children with ASD. Many studies in the literature show increases in activation with cognitive tasks with age, as well as increasing specificity of activation – i.e., less dispersion (Ciesielski, Lesnik, Savoy, Grant, & Ahlfors, 2006; Durston et al., 2006; Passarotti, Smith, DeLano, & Huang, 2007). These effects are generally interpreted as reflecting increasing cortical efficiency over childhood, as cognitive functions become increasingly refined. The fact that this increase with age was only seen in one region with the children with ASD – the middle frontal gyrus – suggests that this typical developmental pattern is disrupted in these children. Overall, these findings provide some evidence in support of the executive dysfunction hypothesis of ASD, suggesting that maturation in key functional areas may be atypical. And again, the finding of atypicalities in age-related change for the ASD group is consistent with evidence from the two other studies showing atypical maturational patterns in structural brain development, which may be underlying these differences in functional maturation.

Evaluating the results of the three studies together, the most striking observation is the repeated finding of a pattern of age by group interactions, which occurs across multiple measures including those of both brain structure and function. This is a critical convergence of the three
studies, as it strongly suggests abnormalities in typical brain maturation in this disorder, evidenced by in some cases reduced, and in other cases increased maturational patterns in the children with ASD. The three studies do not consistently implicate particular brain regions; this, I believe, provides further weight to the idea of ASD as a widely distributed disorder.

It is important to note, however, that the specificity of these results to ASD can only be confirmed through the use of additional control groups, for example, children with developmental disability or other developmental disorders. It is possible that age-related effects would be atypical in other disorders with neurodevelopmental etiologies (e.g., Down’s syndrome) and/or shared genetic underpinnings (e.g., Fragile X). However, the repeated finding of early brain overgrowth (as measured by volume) followed by a period of arrest in ASD has not been reported in any other disorder to date. This particular aspect of neurodevelopmental abnormality appears to be specific to autism, and thus the results of these studies can still be interpreted in light of this well-accepted finding.

It is also important to consider the role of environmental factors that may be implicated in neurodevelopment during the age-range studied (i.e., late childhood/early adolescence). As soon as a baby is born, genetic predispositions interact with post-natal environmental variables such as experience/learning, as well as many other environmental factors later on in development, such as puberty and its associated hormonal changes, which would be particularly important during the age-range studied. It is possible that hormonal changes, while affecting both groups of children, may be affecting children with an ASD differentially, perhaps because of neurological and behavioural abnormalities which have already developed, and contributing in some way to the age-related atypicalities seen in these results. However, as the findings of this study are consistent with literature suggesting an early period of dysregulated brain growth (prior to onset of puberty), I would suggest that it is unlikely that hormonal changes due to puberty provide an alternative explanation to the results.

5.1 Limitations of cross-sectional design

As mentioned in limitation sections of previous chapters, conclusions about developmental trajectories based on these data are limited by the fact that these studies were all cross-sectional in nature. Maturational change with age is best examined by a longitudinal study with a large cohort, where changes with age can be measured within participants, rather than extrapolated
across different participants. It is also possible that the cross-sectional design could interact with phenotypic heterogeneity of the ASD population, perhaps biasing the results in some unanticipated way. Importantly, this would be a limitation of all cross-sectional ASD studies and would not be unique to this work. A longitudinal study will also control for this eventuality. Overall, it is felt that these studies contribute to a body of work that provides the justification and basis for pursuing larger, more resource-intensive longitudinal studies.

5.2 Implications for current models of ASD etiology

Taken together, the three studies provide converging evidence of atypical relations between structural and functional brain measures and age in the ASD population, which supports the growth dysregulation hypothesis (Section 1.3). This model of abnormal cortical maturation holds great promise for understanding the neuropathology of ASD. According to this hypothesis, early overgrowth followed by abnormal slowing disrupts the finely timed balance of induction and termination of gene-regulated growth signals (causing neuronal proliferation and migration) and cell death signals (causing apoptosis), that are necessary for the development of typical anatomical structure and functional connectivity within the brain (Akshoomoff et al., 2002). This leads to anatomical abnormalities, such as previously summarized volumetric abnormalities distributed throughout the brain in both white and grey matter (Section 1.2.1), as well as macro and microstructural grey matter abnormalities in cortical thickness, surface area and morphology and minicolumnar number and spacing (Section 1.2.2).

Importantly, these anatomical changes in turn have profound effects on the development of connectivity within the brain. For example, changes in gyrification are known to affect the development of neuronal connectivity (Armstrong et al., 1995), and increased gyrification seen in ASD (Hardan et al., 2004) may result in the formation of more short distance connections in the brain and impede the formation of longer distance connections. This in turn would lead to reduced large-scale connectivity within the brain, as reflected by the white matter abnormalities seen in this work and other studies. Linking the atypical cortical thickness measures with the DTI results would be an important future direction, to help further elucidate the interactions between these developmental changes in the brain. Abnormalities in minicolumnar spacing and arrangement (Casanova et al., 2002) may also lead to an increase in local connectivity and a relative lack of long distance connectivity (Casanova et al., 2006). It has also been hypothesized
that abnormally increased brain size itself could lead to a similar imbalance in connectivity (Lewis & Elman, 2008), as larger brains encourage short range connectivity, whereas smaller brains engender relatively greater long-range connectivity. Furthermore, a recent study of the development of functional connectivity in typically developing children and young adults revealed that normal development of large-scale brain networks is characterized by a weakening of short-distance connections and a strengthening of long-range cortico-cortical connections (Supekar, Musen, & Menon, 2009). This may be in direct contrast to what is occurring in children with ASD, causing the brain to develop with relative underconnectivity, which has been proposed as an hypothesis of underlying neuropathology in ASD (e.g., Minshew & Williams, 2007). The theory of underconnectivity in ASD, as summarized earlier (Sections 1.2.3 and 1.5.2), is supported by evidence from DTI research (e.g., Barnea-Goraly et al., 2004, Keller et al., 2007; Conturo et al., 2008, Ke et al., 2009), white matter volumetric studies (e.g., Courchesne et al., 2001, Ke et al., 2009, Frazier & Hardan 2009) and functional connectivity analyses (e.g., Just et al., 2007, Kana et al., 2007; Koshino et al., 2008). Underconnectivity would lead to a system that is inefficient for the rapid integration of information from various brain regions. This diminished ability to assimilate sensory and cognitive input would produce significant deficits in complex information processing (Anagnostou & Taylor, 2011).

These effects on connectivity may have a particularly strong impact on the function of the frontal lobes because of their abundance of long association fibres (Casanova et al., 2006). The frontal lobes are the most highly-connected regions of the cortex, with previous studies reporting connections with every other distinct functional area of the brain (Nauta, 1972). It has been suggested that damage to any part of the cortex will either directly or indirectly affect inputs to, and therefore the function of, the prefrontal cortex (Mesulam, 1990). This may underlie the many accounts of frontal dysfunction in autism, and the executive dysfunction hypothesis in general. This is further reinforced by the reports of greater anatomical atypicalities in the frontal lobes (e.g., Carper et al., 2002; Carper & Courchesne, 2005) in children with ASD.

5.3 Broader Implications and future directions

A growing understanding of the neurodevelopmental underpinnings of ASD, and the impact of growth dysregulation, has important clinical applications. In particular, this work and an overall model of early disruption in neurodevelopment, provide further support for the importance of
early intervention in treatment of ASD. This would certainly apply to behavioural intervention, known to be critical in the early years as we know that brain development is a product of gene-environment interaction, and that brain plasticity allows for neurodevelopmental change to occur as a result of learning and environmental exposure through behavioural therapy. Changes in cortical thickness have been reported that are related to skill acquisition across the adult age range (Driemeyer, Boyke, Gaser, Büchel, & May, 2008; Boyke, Driemeyer, Gaser, Büchel, & May, 2008). This further complicates our understanding of what is typical, but does offer a tremendous opportunity for monitoring interventions, as grey matter changes have been reported in specific brain regions that are performance-relevant (Driemeyer et al., 2008; Herdener et al., 2010). White matter tract plasticity has also been associated with skill acquisition (Imfeld, Oechslin, Meyer, Loenneker, & Jancke, 2009), implying that the flat trajectories seen in the present study with DTI measures in children with ASD could potentially be altered with intensive training regimes. Investigation of age-related neuroanatomical changes during a therapeutic intervention is an important future direction of ASD research, to enable investigators to determine whether intervention can alter the neurodevelopmental trajectory in children with ASD, and its impact on the long term behavioural outcomes in the core symptom domains.

Early intervention would also be critical for any future targeted genetic therapies or pharmacological interventions. Genetic research has implicated a number of candidate genes that are important for brain development, such as MET (encodes a receptor tyrosine kinase involved in neuronal growth and organization), Reelin (encodes a protein that controls intercellular interactions involved in neuronal migration and positioning in brain development), neuroligins 3 and 4 (cell adhesion molecules that are important for synaptic maturation and function) and neurexins (binding partners for neuroligins) such as SHANK3 (see Losh, Sullivan, Trembath, & Piven, 2008, for a review). Overall there seems to be growing support for the role of synaptic function genes in autism. But the path from genes to behaviour is an exceedingly complex one. One approach to this problem has been to study biological measures at various stages along the pathway, otherwise known as “intermediate phenotypes” (Giedd et al., 2008). It is thought that intermediate phenotypes should be “closer” to the effects of the genes, and demonstrate higher penetrance (Gottesman & Gould, 2003). It has been suggested that trajectories, rather than static measures, of anatomic brain development could be used as an effective intermediate phenotype in the study of ASD (Giedd et al., 2008). Previous research has shown that the trajectory of
cortical thickness, but not the cortical thickness itself, was predictive of IQ in typically developing children (Shaw et al., 2006), and that trajectories have been shown to be superior to static measures of cerebral volume at discriminating male and female brains (Lenroot et al., 2007). Straightforward relations between function and brain volume are confounded because of research showing inverted U-shaped maturational trajectories of grey matter throughout the cortex (Gogtay et al., 2004), such that at some ages increased cortical thickness is normal, whereas at a later age decreased thickness is more typical. These examples from the study of typical development, and supported by the data from the current thesis, strongly support the idea that trajectories of anatomical brain measures are much better suited to the role of intermediate phenotype than static measures (Giedd et al., 2008).

Such studies will, of course, rely on careful longitudinal research projects, another critical future direction for investigations in the field. The current thesis contributes to the increasing number of neuroimaging studies demonstrating abnormal developmental trajectories in autism, underscoring the pressing need for large scale prospective studies of brain structure and function from very early in life through to adolescence, in order to truly understand the brain-behaviour relations that underlie autism. Additional control groups of children with other developmental disorders will also be necessary to further confirm the specificity of brain findings such as these to the ASD population. Thus, future research with larger numbers, broader age ranges, and carefully selected control groups of children is needed to further explore this developmental disorder. These studies will be essential to better characterize the maturation patterns of the cortex in ASD and gain a more complete understanding of when and how these patterns diverge from that seen in typically developing children, and ultimately, their implications for brain development and mechanisms by which they contribute to ASD symptomatology.
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Appendix 1: Supplemental Material for Chapter 3

Supplemental Figures. Longitudinal, radial and mean diffusivity for 6 additional regions, as reported in Table 3.6, where age by group interactions were seen between the ASD and TD groups.