

Neuropathology of the Social Cognitive Network in Autism

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Abstract

Potential differences in developmental trajectory were investigated in autism at both the macro- and micro-scopic scale, using regional volumetric measurements from in-vivo scans and measurements of minicolumnar organisation of the cortex in post-mortem tissue. In addition, a study was carried out to investigate the sensitivity of measures of cortical diffusion to cortical architecture. Three key regions of interest were studied throughout this thesis, orbital frontal cortex (BA11), primary auditory cortex (BA41) and part of the inferior parietal lobe (BA40).

Subjects with ASD showed increases in grey matter in left parietal cortex and decreases in left BA11 compared to controls. In addition, subjects with ASD showed increased grey matter volume with age in both BA41 and the inferior parietal lobe, whereas controls only showed a negative correlation between grey matter volume in BA41 and age.

Wider minicolumns were found in ASD in all regions, suggesting pathology is not restricted to higher order association areas. Differences seemed more pronounced at younger ages suggesting an altered developmental trajectory in ASD. Such an increase in minicolumnar width arguably underlies the feature-based processing style seen in ASD.

A pilot study using post-mortem DTI scans of MS brains revealed a relationship between measures of the directionality of diffusion and the width of axonal bundles in the cortex, an aspect of the minicolumnar arrangement. When extending this investigation to a set of ASD and control brains, evidence was found for different relationships between axon bundle width and measures of the directionality of diffusion in the cortex, suggesting that although differences in axon bundle width were not seen between groups, there may be differences in the composition of the axon bundles between ASD and control groups.

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Chapter 1: Introduction

Clinical Profile

Autism was first described by Kanner in 1943 (Kanner, 1943) and since then conceptualisations of autism spectrum disorder (ASD) have undergone several transformations. Early theories regarded ASD as ‘childhood schizophrenia’, although changing definitions meant that by 1980 ASD was considered a separate disorder which was biological in origin. Further refinement of the diagnosis, through additions to the criteria, occurred through subsequent editions of the Diagnostic and Statistical Manual (DSM) before the latest reconceptualisation in DSM-5 which reduces the number of subcategories and simplifies the diagnostic criteria (see Table 1.1)(Baker, 2013).

Prior to the publication of DSM-5, ASD had been considered a pervasive developmental disorder, encompassing symptoms in three domains: social interaction, communication and restricted and repetitive behaviours. In DSM-IV ASD was just one of 5 subcategories within the pervasive developmental disorders group, which also included:

- Asperger’s disorder - distinguished by lack of clinical delay in language acquisition
- Rett’s disorder - which has been excluded from DSM-5 due to discovery of the genetic basis
- Childhood Disintegrative disorder

- Pervasive Developmental Disorder – Not Otherwise Specified (PDD-NOS) - a catch-all for those with a pervasive developmental disorder which did not meet criteria for any of the other subcategories

This structure is largely reflected in the International Classification of Diseases (ICD)-10 description of pervasive developmental disorders. Although DSM-5 recognises a lot of the same diagnostic criteria for ASD, it represents more of a conceptual shift, moving from the ‘triad’ of impairments to a definition in terms of two categories: ‘impairment in reciprocal social communication and social interaction’ which combines two previously distinct categories, and ‘restricted, repetitive patterns of behaviour’. There is also increasing emphasis on the presence of sensory abnormalities, something observed in around 75% of patients with ASD (Klintwall et al., 2011) but not previously recognised as part of the diagnostic criteria in the DSM (although it does form part of the ICD-10 criteria).

The other major change in the conceptualisation is the collapse of subcategories of pervasive developmental disorders into the one ‘Autism Spectrum Disorder’, albeit with a more dimensional emphasis revealed through the inclusion of ‘severity levels’ to indicate the degree of support required. This has raised concerns about both patients losing their diagnosis under the new criteria and also the impact on public understanding and sense of community particularly for those who previously had a diagnosis of Asperger’s syndrome (Linton et al., 2014; Volkmar and Reichow, 2013). However, there has been some concern as to whether these subdivisions of PDD are in fact separate disorders, with evidence suggesting Asperger’s syndrome differs from autism quantitatively (e.g. in severity of symptoms) rather than qualitatively (Ozonoff, 2012). This supports a shift towards the more dimensional approach taken by DSM-5, rather than the categorical approach employed by DSM-IV. Almost all studies assessing the

| | ICD-10 | DSM-IV | DSM-5 |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| | Childhood Autism | Autistic Disorder | Autism Spectrum Disorder |
| Age of onset | <p>Presence of abnormal or impaired development before the age of three years in at least one of:</p> <ul style="list-style-type: none"> • Receptive or expressive language as used in social communication • Development of selective social attachments or reciprocal social interaction • Functional or symbolic play | <p>Delays or abnormal functioning in at least one of the following with onset prior to age 3 years:</p> <ul style="list-style-type: none"> • Social interaction • Language as used in social communication • Symbolic or imaginative play | <p>Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities or may be masked by learned strategies in later life)</p> |
| | | A total of six or more from (1), (2) and (3) | |
| Social interaction | <p>Qualitative abnormalities in reciprocal social interaction manifest in at least one of:</p> <ul style="list-style-type: none"> • Failure adequately to use eye-to-eye gaze, facial expression, body posture and gesture to regulate social interaction • Failure to develop (in a manner appropriate to mental age, and despite ample opportunities) peer relationships that involve a mutual sharing of interests, activities and emotions • A lack of socio-emotional reciprocity as shown by an impaired or deviant response to other people's emotions, or lack of modulation of behaviour according to social context, or a weak integration of social, emotional and communicative behaviours | <p>1) Qualitative impairment in social interaction as manifested by at least two of:</p> <ul style="list-style-type: none"> • Marked impairment in the use of multiple nonverbal behaviours such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction • Failure to develop peer relationships appropriate to developmental level • A lack of spontaneous seeking to share enjoyment, interests, or achievements with other people (e.g. by a lack of showing, bringing, or pointing out objects of interest) • Lack of social or emotional reciprocity | <p>Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history</p> <p>(1) Deficits in social-emotional reciprocity, ranging, for example, from abnormal social approach and failure of normal back-and-forth conversation; to reduced sharing of interests, emotions or affect; to failure to initiate or respond to social interactions</p> <p>(2) Deficits in non-verbal communicative behaviours used for social interaction, ranging, for example, from poorly integrated verbal and nonverbal communication; to abnormalities in eye contact and body language or deficits in understanding and use of</p> |

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|---------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Communication | <p>Qualitative abnormalities in communication, manifest in at least two of:</p> <ul style="list-style-type: none"> • A delay in, or total lack of development of spoken language that is <u>not</u> accompanied by an attempt to compensate through the use of gesture or mime as alternative mode of communication (often preceded by a lack of communicative babbling) • Relative failure to initiate or sustain conversational interchange (at whatever level of language skills are present) in which there is reciprocal to and from responsiveness to the communications of the other person • Stereotyped and repetitive use of language or idiosyncratic use of words or phrases • Abnormalities in pitch, stress, rate, rhythm and intonation of speech | <p>2) Qualitative impairments in communication as manifested by at least one of:</p> <ul style="list-style-type: none"> • Delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gestures or mime) • In individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others • Stereotyped and repetitive use of language or idiosyncratic language • Lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level | <p>gestures; to a total lack of facial expressions and nonverbal communication</p> <p>(3) Deficits in developing, maintain and understanding relationships, ranging, for example, from difficulties adjusting behaviour to suit various social contexts; to difficulties in sharing imaginative play or in making friends; to absence of interest in peers</p> |
| Restricted, repetitive and stereotyped behaviours | <p>Restricted, repetitive and stereotyped patterns of behaviour, interests and activities, manifest in at least two of:</p> <ul style="list-style-type: none"> • An encompassing preoccupation with one or more stereotyped and restricted patterns of interest that are abnormal in content or focus, or in one or more interests that are abnormal in their intensity and circumscribed nature although not abnormal in their content or focus | <p>3) Restricted, repetitive and stereotyped patterns of behaviour, interests and activities as manifested by at least one of:</p> <ul style="list-style-type: none"> • Encompassing preoccupation with one or more stereotyped patterns of interest that is abnormal either in intensity or focus • Apparently inflexible adherence to specific, non-functional routines or rituals • Stereotyped and repetitive motor | <p>Restricted, repetitive patterns of behaviour, interests, or activities, as manifested by at least two of the following, currently or by history:</p> <ul style="list-style-type: none"> • Stereotyped or repetitive motor movement, use of objects, or speech (e.g. simple motor stereotypies, lining up toys, or flipping objects, echolalia, idiosyncratic phrases) • Insistence on sameness, inflexible adherence to routines, or ritualised |

| | | | |
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| | <ul style="list-style-type: none"> • Apparently compulsive adherence to specific, non-functional, routines or rituals • Stereotyped and repetitive motor mannerisms that involve either hand or finger flapping or twisting or complex whole body movements • Preoccupations with part-objects or non-functional elements of play materials (such as their odour, the feel of their surface, or the noise or vibration that they generate) • Distress over changes in small, non-functional, details of the environment | <p>mannerisms (e.g. hand or finger flapping or twisting, or complex whole-body movements)</p> <ul style="list-style-type: none"> • Persistent preoccupation with parts of objects | <p>patterns of verbal or nonverbal behaviour (e.g. extreme distress at small changes, difficulties with transitions, rigid thinking patterns, greeting rituals, need to take same route or eat same food every day)</p> <ul style="list-style-type: none"> • Highly restricted, fixated interests that are abnormal in intensity or focus (e.g. strong attachment to or preoccupation with unusual objects, excessively circumscribed or perseverative interest) • Hyper- or hypo-reactivity to sensory input or unusual interests in sensory aspects of the environment (e.g. apparent indifference to pain/temperature, adverse response to specific sounds or textures, excessive smelling or touching of objects, visual fascination with lights or movement) |
| | | | <p>Symptoms cause clinically significant impairment in social, occupations or other important areas of current functioning</p> |

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| Specificity | The clinical picture is not attributable to the other varieties of pervasive developmental disorder; specific developmental disorder of receptive language with secondary socio-emotional problems; reactive attachment disorder or disinherited attachment disorder; mental retardation with some associated emotional or behavioural disorder; schizophrenia of unusually early onset and Rett's syndrome | The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder | These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum disorder and intellectual disability, social communication should be below that expected for general developmental level. |
|-------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

Table 1.1. Diagnostic criteria for autism/autism spectrum disorder according to ICD-10, DSM-IV and DSM-5

correspondence between diagnoses received with DSM-IV and DSM-5 report that not all individuals receiving a diagnosis with DSM-IV will receive a diagnosis with DSM-5, though the rates vary between 42% and 93% (Volkmar and Reichow, 2013). In addition, there is some discrepancy about who will be the most affected, with some studies reporting that the loss of diagnosis is more pronounced in high-functioning individuals, and others reporting that those who previously had a diagnosis of PDD-NOS would be more affected (Volkmar and Reichow, 2013). However, it is important to bear in mind that some of these studies were carried out using draft criteria, and that some of the subjects who lost their ASD diagnosis qualified for a diagnosis of the new social communication disorder, so it is not possible to fully anticipate what the impact of the new DSM-5 criteria will be until they are in use (Volkmar and Reichow, 2013). Additionally, studies assessing the impact of the DSM-5 criteria have used DSM-IV criteria as a 'gold-standard' of assessment to compare against, whereas, if this were the case, no changes to the DSM criteria would have been necessary.

The changing nature of the conceptualisation of ASD and the range of possible criteria that can be met to achieve a diagnosis, highlights the heterogeneous nature of the disorder and the need for understanding of common underlying mechanisms.

Prevalence

A 2012 report by the CDC based on analysis of data from 2008 in 14 sites across the USA found the prevalence of ASDs (autism, PDD-NOS or Asperger Disorder) to be 1 in 88 children aged 8 years (1 in 54 boys and 1 in 252 girls)(CDC, 2012). Other contemporary studies found broadly similar prevalence rates in the USA (ranging from 1 in 91 to 1 in 135, although one study reported rates as high as 1 in 50) (Blumberg et al., 2013; Boyle et al., 2011; Kogan et al., 2009), although reports in the UK have not been

so consistent (ranging from 1 in 88 (Baron-Cohen et al., 2009) through 1 in 162 (Yeargin-Allsopp, 2008) to 1 in 263 for boys and 1 in 1250 for girls (Taylor et al., 2013)). A recent review revealed that results from other European countries showed similar variation in results, although with a similar tendency for more recent studies to report higher prevalence rates (Elsabbagh et al., 2012). It was also found that although an increasing number of studies were being carried out in Japan and China (finding a median prevalence rate of 1 in 769), prevalence rates in the rest of the world have been under-investigated, with few or no studies looking at Africa, South America, Australasia or the rest of Asia (Elsabbagh et al., 2012).

The 2012 CDC report was able to compare its findings with those from previous years, finding an increase of 23% compared to 2006 and an increase of 78% compared to 2002 (CDC, 2012). A number of other studies have also reported increased prevalence of ASD with reports of an 8-fold increase between 1991 and 2001 (Smeeth et al., 2004), an almost 4-fold increase between 1997 and 2008 (Boyle et al., 2011), a 2-4 fold increase between 1990 and 2001 in Scandinavia (Atladottir et al., 2014), and an almost 2-fold increase between 2007 and 2011-2012 (Blumberg et al., 2013). However, there are some studies which suggest a rising prevalence through the 1990s which has since levelled off (Hagberg and Jick, 2010; Taylor et al., 2013) and other studies which find no increase in prevalence at all (Baird et al.; Chakrabarti and Fombonne, 2005).

Several possible explanations have been put forth for this apparent increase in prevalence. A study conducted in the USA found evidence for 'diagnostic switching' whereby part of the apparent increase is due to better diagnosis. Whereas previously individuals might have been classified as having mental retardation or learning difficulties they are now more specifically diagnosed with ASD. Increase in ASD diagnoses was accompanied by decrease in the diagnoses of mental retardation and

learning difficulties (Croen et al., 2002; Shattuck, 2006). In particular a number of changes occurred across the 1990s that may have affected diagnosis rates, changes in diagnostic criteria from DSM-III to DSM-IV were found to produce a 1.4-fold increase in the number of diagnostic cases and that there was also an increase in the detection of true autism cases in the population (Wazana et al., 2007). At the same time there was also a decrease in age of diagnosis, with a study in California finding a drop from a mean age of diagnosis of 6.9 years among children born in 1987 to 3.3 years among children born in 1994 (Croen et al., 2002). In a model attempting to investigate the effects of changes in diagnostic criteria, age of diagnosis and better detection of true autism cases on prevalence rates, Wazana et al. (2007) found that the most conservative model combining these factors predicted a 2.4-fold increase in prevalence per calendar year. This model assumes independence of all three factors which may have led to an overestimate of the predicted increase, but at the same time only three factors have been modelled which may have led to an underestimate of the combined effect of changes in diagnosis on estimates of prevalence. It has also been found that ASD is not the only disorder to have shown an apparent increase in prevalence over the last 20 years, similar increases have been seen in hyperkinetic disorder, Tourette's syndrome and obsessive-compulsive disorder, which the authors argue is evidence for a largely non-etiological basis for this increase, suggesting it may instead be due to better identification and diagnosis of ASD (Atladottir et al., 2014).

It has also been suggested that differences in methodology of the studies may have led to both widely varying estimates of prevalence and findings of increasing prevalence. Fombonne (2009) notes that when there have been multiple studies conducted at the same time point within the same country, studies which use intensive population-based screening find higher estimates of prevalence than studies that use more passive

administrative methods to identify cases. Factors such as geographical location within a country have also been found to have an effect, with the lowest prevalence rates in the UK being found in Wales and the highest in the South East of England (Smeeth et al., 2004). Similarly the 2012 CDC report found prevalence rates to vary across the USA from 1 in 208 in Alabama to 1 in 47 in Utah (CDC, 2012). Such variation suggests there may be methodological differences in the data collection, and both these studies used information gathered over multiple sites. In the UK study, cases were identified through records from between 34 and 557 GP practises and in the USA cases were identified and assessed at different sites across 14 US states. Additionally a positive association between socioeconomic status (SES) and ASD diagnoses has been found in the USA, which was stronger in cases with a pre-existing ASD diagnosis, although still present in those without a pre-existing diagnosis (Durkin et al., 2010). This suggests that although some of the contribution of SES to ASD prevalence is independent of ascertainment bias, there is still an effect of ascertainment bias, which in the USA in particular, could be associated with access to healthcare. The impact of geographical variation in sites participating in national surveys of this kind could therefore affect estimates of prevalence rates, with the CDC report finding that the inclusion of an additional county to the North Carolina site in 2008 resulted in a 15% increase in overall ASD prevalence compared to when this county was excluded (due to the very large effect of this particular region it was excluded from comparisons with previous years, however other sites also had variations in the geographical areas covered between the years examined) (CDC, 2012). The only study to have employed strictly identical criteria for identification of cases in the same geographical area in the UK found no difference in prevalence rates of pervasive developmental disorders between 1992-1995 and 1996-1998 (Chakrabarti and Fombonne, 2005).

Gender differences

ASD has been repeatedly found to show a stronger gender bias with many studies finding a ratio of approximately 4 males to every female (Blumberg et al., 2013; Fombonne, 2009; Volkmar et al., 1993), although findings range from a male:female ratio of 1.33:1 to 15.7:1 (Fombonne, 2009). The study finding the largest ratio of 15.7:1 also had a sample with one of the highest proportions of subjects with normal IQ (60%) out of those for which this figure was available (Fombonne, 2009), which is consistent with the idea that females with ASD tend to have lower IQ and so are particularly under-represented at higher IQ ranges (Kirkovski et al., 2013; Lord et al., 1982; Volkmar et al., 1993). IQ differences have not always been found (Mandy et al., 2012), although in the light of the methodological and social factors that may affect gender specific diagnosis that will be discussed later, it is interesting to note that it is a more recent study which finds this. There is some evidence for a genetic basis to IQ dependent male:female ratios. One study comparing probands from simplex and multiplex families found an approximately constant ratio of 2:1 across all IQ groups in multiplex families, whereas in simplex families the ratio varied from 1:2.7 in a below 50 IQ group, to roughly 1:1 for those with IQ between 50 and 70 and 8.3:1 for those with IQ over 70 (Banach et al., 2009).

There have been two main approaches to attempting to explain the gender ratio seen in autism, one focusing on methodological and diagnostic issues which may cause a bias towards an artefactually increased prevalence in males, and one focusing on underlying biological and genetic differences which could cause a true prevalence increase in males; although the two are not mutually incompatible. Rutter et al. (2003) argues that in order to establish that there truly is a sex-difference in the presentation of psychological

conditions there are a number of methodological issues which must first be addressed. Sex comparisons should be based on a representative sample from the general population (Rutter et al., 2003), whereas in autism many studies have relied on samples from clinical groups which tend to have a disproportionate representation of males (Kreiser and White, 2013). The data sources used for case identification may affect the results (Rutter et al., 2003) and most studies of ASD have relied on educational and health records, where boys might be more likely to be identified due to a more disruptive presentation of their symptoms (Kreiser and White, 2013). There may also be an expectation bias in clinicians resulting from the conceptualisation of ASD as a condition which is much more prevalent in males, and there is some evidence from population based studies to suggest that for similar levels of social impairment clinicians are less likely to diagnose females with ASD (Kreiser and White, 2013). Finally, there may be a different presentation of the same condition in males and females; and the diagnostic criteria may be more representative of the manifestation in one gender in particular (Rutter et al., 2003). This would lead to under-representation of the less well characterised gender, as has been found in conduct disorder and ADHD (Kreiser and White, 2013). Indeed it has been found that there are sex differences in scores on questionnaires of autistic traits in the general population, suggesting items included on these questionnaires are more representative of male behaviour in general (Williams et al., 2008).

A number of studies have characterised sex differences among those with an ASD diagnosis, finding females show better executive function (Bölte et al., 2011), better communication (Hartley and Sikora, 2009; McLennan et al., 1993), fewer restricted and repetitive behaviours (Bölte et al., 2011; Hartley and Sikora, 2009; Kirkovski et al., 2013; Lord et al., 1982; Mandy et al., 2012), lower visual spatial performance (Bölte et al., 2011), more social problems, particularly at adolescence and older ages (Carter et al.,

2007; Holtmann et al., 2007; McLennan et al., 1993), more severe impairments in developing friendships (Kirkovski et al., 2013), more internalising symptoms (Solomon et al., 2012) including higher levels of anxiety and depression (Hartley and Sikora, 2009; Werling and Geschwind, 2013), higher avoidance of demands (Kopp and Gillberg, 2011), and generally poorer outcomes in adulthood (Howlin et al., 2004); whereas males were found to have more externalising behaviours such as hyperactivity/inattention, aggressive behaviour and reduced prosocial behaviour (Mandy et al., 2012; Werling and Geschwind, 2013). This pattern of presentation, particularly the tendency towards more passive and internalising symptoms in females as opposed to the externalising behaviours shown by males, suggests cases in males may be recognised more often, or earlier, due to the more disruptive nature of their symptoms (Kreiser and White, 2013) and could mean only the more extreme female cases are detected, leading to artificially inflated findings of more severe symptomatology in females.

Gender specific socialisation could also have an impact on rates of diagnosis in females. Parents are known to treat boys and girls differently, and a more intimate relationship between mothers and daughters may help to improve their language and empathising abilities (Kreiser and White, 2013) which may help them to mask their social deficits (Dworzynski et al., 2012; Goldman, 2013). In addition, expectations of gender-appropriate behaviour may influence the interpretation of symptoms in girls, with social deficits being interpreted as 'shyness' (Goldman, 2013; Kreiser and White, 2013). Similarly, a parental expectation for females to show more social behaviour might be responsible for reports of more severe social problems in females (Holtmann et al., 2007).

Although there are likely methodological issues in diagnosis leading to under-representation of the true rate of ASD in females, there may still exist biological or

genetic differences between genders, especially given the different patterns of symptomatology that have been identified. Such a gender bias initially led researchers to look for an X-chromosome linked genetic explanation. Although a few ASD candidate genes have been identified on the X-chromosome (including FMRP, MECP2, NLGN3 and NLGN4X), these are not sufficient to explain the majority of cases and genetic inheritance of ASD does not follow the typical X-linked pattern (Werling and Geschwind, 2013). Alternative genetic explanations suggest that females have a higher threshold for reaching affectation status, that is, it takes more mutations before they display the ASD phenotype. This model predicts that as female probands are carrying a higher genetic load, the relatives of female probands should be more at risk than the relatives of male probands, but findings relating to this are mixed (Werling and Geschwind, 2013).

Other biological explanations have focused on Baron-Cohen's (Baron-Cohen, 2003) influential 'extreme male brain' theory, which will be discussed in more detail later, but essentially suggests that autism is an extreme presentation of the normal male phenotype. This has been extensively linked with the idea that as prenatal testosterone is important in determining development of the foetus as a male; higher levels of prenatal testosterone may also be important in the development of autistic traits and ASD itself. As it is often difficult and intrusive to obtain measurements of prenatal testosterone, the ratio of the second finger to the fourth finger (2D:4D) has often been used as a proxy as it has been shown to relate to prenatal testosterone levels (Geier et al., 2012). Lower 2D:4D ratios have been found in ASD and first degree relatives, and in typically developing children it has been found to relate to autism traits such as frequency of eye contact and quality of social relationships (Geier et al., 2012). Testosterone has been found to decrease, and oestrogen increase, expression of a candidate ASD gene, RORA, which acts as a

regulator of aromatase, an enzyme involved in converting testosterone to oestrogen (Sarachana et al., 2011). Analysis of post-mortem tissue also found levels of aromatase to be reduced in the frontal cortex of subjects with ASD (Sarachana et al., 2011). Findings from *staggerer* mice which have only one functional copy of the RORA gene, showed a gender dependent effect of the mutation on Purkinje cell loss (one of the most consistent neuropathological observations in ASD) where male mice showed an earlier onset of Purkinje cell loss (Doulazmi et al., 1999).

Genetics

ASD is generally considered to have a strong genetic basis with heritability estimates ranging from 70-90% (Bailey et al., 1995; Freitag, 2006; Geschwind, 2011), although some recent studies have suggested lower estimates (Hallmayer et al., 2011; Skuse, 2007). Investigation of the three components of ASD (social impairment, communication impairment and restricted and repetitive behaviours) revealed that all three components were individually highly heritable but showed low covariation, suggesting these features are inherited independently of one another (Ronald et al., 2006).

Approximately 10% of ASD cases are syndromic, that is due to a monogenetic syndrome that has phenotypic overlap with ASD. In contrast, the genetic basis of idiopathic ASD has so far remained elusive with the question of whether it is more likely to be the result of common genetic variants in the population or rare alleles still hotly debated (El-Fishawy and State, 2010). As of March 2014, the SFARIgene website (<https://gene.sfari.org>), an online database of genes implicated in ASD susceptibility, listed 604 genes in which mutations had been reported, 3652 of which were rare variants and 878 of which were common variants.

Syndromic ASD

Fragile X syndrome (FXS) is caused by an expansion of a CGG repeat (>200 repeats) in the 5' untranslated region of the FMRI gene on the X chromosome (Bagni and Greenough, 2005). FXS is responsible for 2-6% of all cases of ASD, with 30% of males with FXS reaching criteria for autism and an additional 30% having pervasive developmental disorder – not otherwise specified (Hagerman et al., 2010). Higher rates of ASD are also seen in carriers of the premutation (between 50 and 200 repeats) with estimates ranging from 14-19% for males and 1-5% of females (Hagerman et al., 2010).

Fragile X mental retardation protein (FMRP) which is encoded by the FMRI gene is a RNA-binding protein involved in many aspects of mRNA metabolism and has roles in regulation of the mTOR pathway, cytoskeleton structure and function, synaptic formation, transmission and plasticity (Bagni and Greenough, 2005; Hagerman et al., 2010). In the absence of FMRP long, thin and immature dendritic spines are observed, which may reflect an abnormal pruning process (Bagni and Greenough, 2005), as well as migration problems of neurons in the hippocampus and cerebellum similar to those observed in ASD (Hagerman et al., 2010).

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder resulting from mutations in either TSC1 or TSC2. TSC has a wide range of symptoms including epilepsy, mental retardation, dermatological manifestations and cortical tubers (Crino et al., 2006). Reports indicate ASD occurs in approximately 25% of those with TSC (Gutierrez et al., 1998).

TSC1 and TSC2 are tumour suppressor genes, the products of which form a complex which works to regulate the mTOR pathway, important in regulation of cell growth and proliferation (Crino et al., 2006). Recent work in mice has linked lack of TSC1 function

in cerebellar purkinje cells to social impairment, restrictive behaviour and abnormal vocalisations indicating impairment in the three core domains seen in ASD (Tsai et al., 2012).

Chromosomal abnormalities

Autism has been associated with a high occurrence of chromosome abnormalities (Marshall et al., 2008) including translocations, inversions and copy number variants (CNVs). CNVs are segments of DNA, ranging in size from 50 base pairs to several megabases, which vary between individuals in the number of copies present, due to deletion, duplication or insertion; and have been suggested to occur at increased frequency in autism as compared to controls (6-10% vs. 1-3%) (Persico and Napolioni, 2013). However, it has increasingly become apparent that the location of the CNV may be more important than the overall CNV burden (Persico and Napolioni, 2013), with network analyses of genes affected by rare CNVs finding these loci to be involved in synapse development, axon targeting and neuron motility (Gilman et al., 2011).

De Novo

Recent work investigating the contribution of CNVs to ASD has increasingly focussed on the role of de novo CNVs, that is mutations that have not been inherited from either parent (Murdoch and State, 2013). De novo CNVs have been found to be particularly overrepresented in simplex families (i.e. those with only one affected child) as compared to multiplex families (i.e. those with more than one affected individual), with duplications at 7q11.23 (deletions of this region is known to cause Williams Syndrome), and both duplications and deletions at 16p11.2 emerging as particularly associated with ASD (Sanders et al., 2011).

In addition to looking for large scale changes on the order of CNVs, the advent of whole genome sequencing and whole exome sequencing (WES) has allowed de novo mutations to be investigated at the level of point mutations. Initial WES studies identified a number of de novo mutations; the majority of which increased risk, but only by a small amount, and not significantly to be able to be said to ‘cause’ ASD. Many of the genes identified in these studies were found to be involved in a small number of gene networks, such as those involved in synaptic plasticity and β -catenin/chromatin remodelling (Murdoch and State, 2013; Persico and Napolioni, 2013).

Common Variants

While the approaches described above have focussed on identifying genes or regions of the chromosome that show mutations in ASD, another approach is to focus on the role of common variants. Common variants are alleles which exist in the population at relatively high frequencies (>1%) and contribute to risk for the disorder or condition (Becker, 2004). Genome-wide association studies, which look at how frequently particular single-nucleotide polymorphisms (SNPs) occur in the population of interest compared to controls, are the most commonly used method for identifying common variants. While many different common variants associated with autism have been identified, the extent to which these findings have been replicated varies, with some of the most consistently replicated genes including SLC6A4 (a serotonin transporter), GABRB3 (a GABA receptor), ITGB3 and OXTR (involved in cell signalling), CNTNAP2 (a protein found at the synapse) and EN2, MET and RELN (all of which are involved in development of the central nervous system) (Persico and Napolioni, 2013).

Common pathways

As is becoming apparent, a large number and variety of genes have been implicated in ASD. Recent attempts to identify unifying concepts have focussed on possible pathways that may be disrupted by genetic mutations at a number of levels. An analysis of regional patterns of gene expression identified two modules of genes that were differentially expressed in ASD compared to controls, downregulation of a module related to synaptic function and upregulation of a module involved in immune response (Voineagu et al., 2011). This has led to suggestions that two pathways are involved in ASD; one involving cellular and synaptic growth (associated with dysregulation of the mTOR pathway and mutations in TSC1/2 and FMR1) and one involving an imbalance between excitation and inhibition (associated with mutations in NLGN, SHANK and NRXN)(Bourgeron, 2009).

Two recent studies have looked at huge numbers of candidate genes in the context of early cortical development to identify modules of genes that may be affected in autism. From analysis of expression of over 15,000 genes from 8 weeks after conception to 1 year of age, Parikshak et al. (2013) identified modules of genes whose expression was synchronised. Several of these modules were found to be enriched in ASD candidate genes, particularly modules involved in early transcriptional regulation and synaptic development. Several of these modules were found to be regulated by FMRP, which has also been implicated in ASD through its link to Fragile X Syndrome. Analysis of the localisation of these genes revealed that there is enrichment of these ASD genes in glutamatergic neurons in upper cortical layers. Another study by Willsey et al. (2013) also looked at networks of genes associated with known ASD candidate genes during foetal development. This study focused on nine genes with strong evidence for association with ASD, and then identified networks of other genes that were expressed at

similar times and locations in the brain. Networks that included disproportionately high numbers of other genes that have been implicated in ASD (other than the nine they started with) were found to be expressed 10-24 weeks after conception and contained genes relating to layer 5/6 glutamatergic neurons. Although relatively few studies have been conducted so far attempting to relate ASD candidate genes into networks such as these, already these studies are beginning to show the incredible amount of convergence between pathways involving ASD candidate genes. Indeed, these two recent studies alone independently implicate glutamatergic neurons in ASD, and highlight the importance of considering the developmental time course of changes that might be going on in ASD.

Further evidence for the importance of taking developmental stage into consideration comes from a study by Chow et al. (2012). This looked at gene expression patterns in regions of prefrontal cortex. In cases aged below 14 years affected genes were found to be those involved in determining cell number, cortical patterning and differentiation, whereas in adults the genes affected were those involved in signalling and repair.

Another unifying concept that has begun to emerge is the possibility of reduced differentiation between different regions of the brain in ASD. Voineagu et al. (2011) found attenuation of the regional difference in gene expression between frontal and temporal cortex in ASD. Similarly, a study looking at expression of genes in the prefrontal cortex and cerebellum found a smaller number of genes differentially expressed between the regions in ASD than in controls (322 vs. 2,000) (Ziats and Rennert, 2013). This is consistent with the suggestion, based on imaging studies, that there is less specialisation of regions of the brain in ASD (Minshew and Keller, 2010). However, a similar attenuation between gene expression in occipital cortex and in

cerebellum was not found (Ginsberg et al., 2013), suggesting such attenuation may be specific to certain regions of the brain.

Given the genetic heterogeneity evident in ASD and the increasing emphasis on many genes affecting common pathways, it may be profitable to look instead at the structural and functional differences these may give rise to. In particular the suggestions of less heterogeneity between brain regions may also be evident at the microstructural level.

Volumetric findings

One of the most consistent volumetric findings in ASD has been of increased brain size (Stanfield et al., 2008). Kanner (1943) noted increased head size in five of the eleven children described in his original report. However, more recent studies of data across the age range have suggested this may not reflect an absolute difference so much as an altered developmental trajectory (Courchesne et al., 2011a). A meta-analysis of brain volume data in ASD revealed brain sizes tend to be smaller than controls at birth, but begin to increase shortly afterwards resulting in larger brains in ASD during childhood (Redcay and Courchesne, 2005). The rate of growth in ASD seems to slow during childhood, allowing controls to catch up, resulting in little difference in brain volume by adolescence (Redcay and Courchesne, 2005). Analysis of the distribution of these volumetric increases suggest that they are not equal across the brain, but are more pronounced in frontal regions (Carper et al., 2002; Herbert et al., 2004), with Herbert et al. (2004) noting that it is the later myelinating areas that show the largest increases compared to controls.

More localised volumetric differences have also been reported in ASD, though this have been much more mixed than those found for overall brain volume. Some of the more consistent findings have been of reduced volume of the corpus callosum and increased

volume of the caudate and amygdala (Brambilla et al., 2003; Stanfield et al., 2008). These findings will be discussed in greater detail later (Chapter 2).

A number of studies have reported localised differences in the cortex in ASD, with altered cortical thickness found in a range of areas including frontal regions (Casanova et al., 2013; Chung et al., 2005; Ecker et al., 2014; Hadjikhani et al., 2006; Hyde et al., 2010; Jiao et al., 2010; Raznahan et al., 2010; Zielinski et al., 2014), parahippocampal gyrus and fusiform (Ecker et al., 2010; Raznahan et al., 2010), temporal regions (Chung et al., 2005; Hadjikhani et al., 2006; Hardan et al., 2006; Hyde et al., 2010), and inferior parietal lobe (Ecker et al., 2010; Hadjikhani et al., 2006; Hyde et al., 2010; Raznahan et al., 2010; Zielinski et al., 2014), although differences have not been found in all studies (Casanova et al., 2009). In addition, the direction of these findings have been mixed with some studies finding increases in cortical thickness (Ecker et al., 2010; Hardan et al., 2006) and others decreases (Casanova et al., 2013; Chung et al., 2005; Ecker et al., 2010; Hadjikhani et al., 2006; Jiao et al., 2010; Scheel et al., 2011). Although findings of increases in cortical width would be consistent with findings of reduced minicolumnar width (Harasty et al., 2003), as has been reported in ASD (Buxhoeveden et al., 2006; Casanova et al., 2006a; Casanova et al., 2002b; Casanova et al., 2002c; Casanova et al., 2006b), typically those studies which have reported increases in cortical width have not measured surface area, decreases in which typically accompany increases in cortical thickness (Harasty et al., 2003). In fact the only studies to do so have reported either no difference in surface area (Ecker et al., 2010) or decreased cortical thickness accompanied by decreased surface area (Ecker et al., 2014).

Three recent studies have again highlighted the importance of taking age into account, finding that the direction of difference in cortical thickness is age dependent (Ecker et al., 2014; Raznahan et al., 2010; Zielinski et al., 2014). Although the studies by Ecker et al.

(2014) and Zielinski et al. (2014) identify a group by age interaction in cortical thickness in some of the same areas (postcentral gyrus bilaterally and rostral middle frontal gyrus); this interaction goes in different directions in each study (but is consistent within a study). Ecker et al. (2014) found several regions of reduced cortical thickness in ASD, but of those, the regions which showed a significant interaction with age showed reduced cortical thickness in childhood and increased cortical thickness in adulthood, which is consistent with the findings of Raznahan et al. (2010). In contrast, Zielinski et al. (2014) found significant group by age effects whereby thicker cortex was seen in childhood and thinner cortex in adulthood (with the trajectories crossing at around 15 years of age). The different findings of these two studies are hard to reconcile given the very different relationships they find, in several cases in the same regions, while both include subjects across the age at which they expect the trajectories of cortical thickness change to cross. The study by Zielinski et al. (2014) does include subjects over a wider age range (3-39 years vs. 7-25 years) which may account for the fact that all regions showing a significant relationship between age and cortical thickness showed a quadratic relationship, rather than the mix of quadratic and linear relationships seen by Ecker et al. (2014). In addition, both use similar imaging parameters and data analysis methods (i.e. use of Freesurfer to derive cortical surface models), however Zielinski et al. (2014) use both right and left handed participants, and include 15 ASD subjects with IQ scores below 70, whereas Ecker et al. (2014) restricted their sample to high functioning (IQ greater than 70) right-handed individuals; suggesting that perhaps the effect of these factors on cortical thickness may merit further investigation. This developmental framework may also help to clarify some of the previous discrepant findings, as the three studies looking just at adults report decreased cortical thickness (Chung et al., 2005; Hadjikhani et al., 2006; Scheel et al., 2011), and in their study of children between 8 and

12 years, Hardan et al. (2006) reported increased cortical thickness (although in a study of 6 to 15 year olds Jiao et al. (2010) reported decreased cortical thickness). In a 30 month follow up of their original study Hardan et al. (2009a) no longer found differences in cortical thickness, but demonstrated an accelerated rate of cortical thinning in ASD subjects compared to controls. This suggests that cortical thickness may follow a similar developmental trajectory to that seen in total brain volume, where there is an initial increase in ASD, followed by a period of decline such that cortical thickness becomes thinner in ASD in adults.

In addition to gross volumetric differences, more subtle differences in geometric features have been reported. For example there have been reports of increased sulcal depth in ASD in the intraparietal sulcus and regions of the superior frontal cortex (Ecker et al., 2010); alterations in degree of cortical folding in the inferior parietal lobe, post-central gyrus and orbitofrontal regions (Ecker et al., 2010); changes in cortical shape in the sylvian fissure, superior temporal sulcus, intraparietal sulcus, inferior frontal gyrus (Amaral et al., 2008) and pars opercularis (Nordahl et al., 2007); and shifting of a number of sulci (Levitt et al., 2003). Nordahl et al. (2007) reported more prominent differences in intraparietal sulcal depth, and shape of the pars opercularis in younger (7.5-12.5 years), compared to older (12.5-18 years), children with ASD. This again suggests early abnormalities which ‘normalise’ with age, although it would have been interesting for the authors to investigate whether this was due to accelerated development of the ASD group along the normal trajectory with the typically developing children catching up later; or whether it was due to abnormal development in the ASD group which then altered to rejoin the normal trajectory. These subtle geometric changes are of interest, not only because they could act as confounding factors complicating identification of ROIs in ASD and the quality of registration between ASD and control MRI scans, but also

because it has been suggested that such alterations in geometry and cortical folding could arise from abnormal connectivity between areas (Van Essen et al., 2006).

Structural Connectivity

Volume, or cross-sectional area, of the corpus callosum has been used as an indicator of the degree of inter-hemispheric connectivity (Jancke et al., 1999; Jäncke et al., 1997), with reductions in callosal area being found in ASD (Boger-Megiddo et al., 2006; Egaas et al., 1995; Frazier et al., 2012; Frazier and Hardan, 2009; Freitag et al., 2009; Hardan et al., 2000; Hardan et al., 2009b; Keary et al., 2009; Piven et al., 1997; Vidal et al., 2006). Although studies disagree as to where the differences are located with some studies reporting that changes are restricted to, or more pronounced in, more anterior (Frazier and Hardan, 2009; Hardan et al., 2000), or posterior regions of the corpus callosum (Egaas et al., 1995; Freitag et al., 2009). Several studies have linked smaller corpus callosums to more severe the ASD symptomatology (Boger-Megiddo et al., 2006; Hardan et al., 2009b; Prigge et al., 2013) and reduced performance on cognitive tasks (Keary et al., 2009; Prigge et al., 2013). In fact, several recent studies have suggested increased rates of ASD traits in callosal agenesis (a condition where the corpus callosum fails to develop) (Badaruddin et al., 2007; Lau et al., 2013; Paul et al., 2014).

One technique that can be used to investigate aspects of connectivity in-vivo is diffusion tensor imaging (DTI). DTI is a form of magnetic resonance imaging (MRI) that allows detection of structure by looking at the diffusion of water. In the absence of any structure (e.g. cell membranes), the diffusion is unrestricted and so equally likely in all directions. This is known as isotropic diffusion. In the presence of coherent structure, such as axonal membranes, diffusion will be restricted and anisotropic, that is directional. By following the direction of the diffusion, white matter tracts of the brain can be reconstructed, and

parameters such as fractional anisotropy (FA) and mean diffusivity (MD) can be used to probe the microstructural organisation of these tracts. Although diffusion in the grey matter is not isotropic, it shows much lower anisotropy than the white matter, and consequentially most studies to date have focused on using DTI to investigate white matter tracts in ASD.

The majority of DTI studies have reported reduced FA in ASD either at the whole brain level (Shukla et al., 2010) (although several others have found no difference in mean white matter FA (Ameis et al., 2011; Groen et al., 2011; Jou et al., 2011a)), in specific white matter regions (Barnea-Goraly et al., 2004; Cheung et al., 2009; Ke et al., 2009; Lee et al., 2007; Noriuchi et al., 2010; Thakkar et al., 2008) or associated with specific white matter tracts (Alexander et al., 2007; Barnea-Goraly et al., 2010; Brito et al., 2009; Cheon et al., 2011; Conturo et al., 2008; Jeong et al., 2011; Jou et al., 2011a; Jou et al., 2011b; Keller et al., 2007; Kumar et al., 2010; Langen et al., 2012; Lo et al., 2011; Pardini et al., 2009; Poustka et al., 2012; Sahyoun et al., 2010a; Sahyoun et al., 2010b; Shukla et al., 2010; Shukla et al., 2011a).

Although the majority of studies investigate changes in FA, differences have also been found in a number of other measures. Differences have been reported in the shape of the uncinate fasciculus (Kumar et al., 2010) and in frontal lobe tracts, with higher curvature being found in ASD (Jeong et al., 2011). In addition, differences in the 'skewness' of the diffusion tensor (which reflects how prolate or oblate it is) in the superior temporal gyrus and temporal stem were able to discriminate subjects with ASD from typically developing controls (Lange et al., 2010).

Several studies have attempted to specifically address the issue of whether there is underconnectivity at the global level but overconnectivity at the local level. One study

separated out long-distance white matter tracts from short-distance white matter tracts (those less than 35mm long) and found reduced FA for only the long-distance tracts, suggesting altered microstructure of just those fibres (Shukla et al., 2011b). However, an earlier study focusing just on the frontal lobe found reduced FA in short-range fibres and no difference in FA in long-range fibres. Although they found the ASD participants showed a different distribution of fibre lengths compared to typically developing controls, there was no evidence for a greater amount of short-range connectivity in ASD (Sundaram et al., 2008). There may be several possible reasons for this difference in findings, one of which may be due methodological differences. The studies differ on both the slice thickness used, which influences the size of structures that can be reliably detected, and on the b-value used, which can influence the ability of DTI to detect some features (for example the ability to distinguish between kissing and crossing fibres) (Travers et al., 2012). However, another difference between these two studies is in the age of the participants. In the study by Sundaram et al. (2008) the mean age of the participants was 4.8 years, whereas in the study by Shukla et al. (2011b) the mean age of participants was 12.6 years (range 9-18 years).

A number of other studies have demonstrated an altered relationship between age and DTI measures in ASD compared to typically developing controls. Positive correlations between FA and age seen in controls have been found to be absent or much weaker in ASD (Alexander et al., 2007; Cheng et al., 2010; Lee et al., 2007; Shukla et al., 2011a; Shukla et al., 2011b), although one study has shown a positive correlation in ASD in the right posterior limb of the external capsule whereas a negative correlation is seen in controls (Keller et al., 2007).

This general lack of correlation between age and FA increases in ASD is consistent with the idea of earlier development in ASD followed by an arrest of growth during the time

development would usually be occurring. This is also consistent with the findings from a few studies that, unlike the studies described so far that have predominantly been conducted in older children, adolescents or adults, have been conducted in very young children, typically under the age of 6 years. For example, a study by Ben Bashat et al. (2007) found that children with ASD between the ages of 1.8-3.3 years showed a significant increase in the proportion of pixels in white matter which showed 'mature' FA values. They also found higher FA in children with ASD in five white matter pathways, mainly in the left hemisphere but also in the corpus callosum, and significantly lower FA in only the left cortico-spinal tract. Although this study has been criticised for its use of a high b-value, which is thought to reflect intracellular diffusion properties rather than the extracellular diffusion revealed by conventional DTI imaging (Groen et al., 2011), the finding of increased FA at very young ages has also been found in other studies using standard b-values (Weinstein et al., 2011; Wolff et al., 2012). Wolff et al. (2012) tracked changes in FA in infants at high-risk for ASD over the first two years of life, scanning them at 6,12 and 24 months, with ASD status assessed at 24 months using the ADOS. They found higher FA in several regions, including the corpus callosum, at 6 months in the ASD-positive group, although by 24 months it was the ASD-negative group that showed higher FA in two regions. This shows the importance of interpreting these findings in a developmental context and shows that the patterns present at very early stages of development may be different to those observed later. It should be noted, however, that several other studies have noted some areas of increased FA in ASD in older, but pre-adolescent children (Brito et al., 2009; Cheung et al., 2009; Ke et al., 2009; Sahyoun et al., 2010a; Sivaswamy et al., 2010), with one study finding this in adolescence (Cheng et al., 2010); although these studies also found areas of reduced FA, and in most cases more regions were found with reduced FA than increased FA. In

addition, the study by Wolff et al. (2012) found increased FA only at 6 months, whereas the other two studies finding increased FA looked at children aged between 1.5 and 6 years (Ben Bashat et al., 2007; Weinstein et al., 2011). However, by only following up ASD status at 24 months Wolff et al. (2012) may have missed some children who later go on to develop ASD, and by looking at only children who were at high risk for developing ASD they may have been detecting smaller differences than would have been seen if they had been compared to a low risk group. Evidence for the latter possibility is provided by a study by Barnea-Goraly et al. (2010) which found similar changes in white matter pathways in children with autism and their unaffected siblings when compared to typically developing controls. Therefore, the developmental time course of these diffusion-based measures in ASD compared to typical development, particularly at very early ages, remains an important area of investigation.

Functional Connectivity

Functional connectivity has been defined as ‘the observed temporal correlations between spatially remote neurophysiological events’ (Friston et al., 1993). Therefore, functional connectivity analysis looks for co-activations of different regions of the brain, either during a task to identify abnormal functional connectivity between regions involved in task performance, or during a resting-state scan, where the participant is not required to do anything except lie still in the scanner (although this is sometimes operationalised as the rest periods between blocks of tasks).

Reduced functional connectivity in ASD has been reported in studies looking at sentence comprehension (Just et al., 2004; Kana et al., 2006), face processing (Kleinhans et al., 2008; Welchew et al., 2005), attribution of mental states to animated shapes (Castelli et al., 2002), inhibitory control (Agam et al., 2010; Kana et al., 2007; Perez Velazquez et

al., 2009), working memory (Koshino et al., 2005; Koshino et al., 2008), visuomotor (Villalobos et al., 2005) and motor tasks (Mostofsky et al., 2009), as well as in response to auditory stimuli in an MEG study (Wilson et al., 2007).

There are two main approaches that can be taken for analysis of resting state data, an ROI driven approach, looking at functional connectivity between one, or a few, areas of the brain, and the rest of the brain; or a more hypothesis-free whole brain approach, looking at all patterns of functional connectivity across the whole brain. Although the majority of studies report underconnectivity in ASD, such methodological differences may go some way to explaining the number and variety of connections which have been reported to show underconnectivity. For example reduced functional connectivity has been reported in the ‘social’ network (Gotts et al., 2012; von dem Hagen et al., 2013), the ‘task negative network’ (which is associated with social and emotional processes) (Kennedy and Courchesne, 2008), the dorsal attention network (Mueller et al., 2013), the default mode network and its sub-networks (Assaf et al., 2010; Mueller et al., 2013; Washington et al., 2013; Weng et al., 2010), relating to the insula (Ebisch et al., 2011), voice-selective regions of cortex (Abrams et al., 2013), anterior and posterior regions of the brain (Cherkassky et al., 2006) and between hemispheres (Anderson et al., 2011), particularly regions associated with language processing (Dinstein et al., 2011). In addition, there have been attempts to link levels of functional connectivity between specific regions with different aspects of the ASD phenotype, although this has yielded relationships with a number of different functional connections for each aspect of the phenotype, for example higher scores on the ADOS and ADI social sub-scales alone have been related to lower functional connectivity between the precuneus and frontal cortex (Assaf et al., 2010), reduced connectivity between the posterior cingulate cortex and right parahippocampal gyrus (Monk et al., 2009), superior frontal gyrus, temporal lobes and parahippocampal

gyri (Weng et al., 2010) and connectivity in the right posterior parahippocampal gyrus, left temporal pole and left lingual gyrus (Lynch et al., 2013).

This preponderance of findings of functional underconnectivity has largely been viewed as support for an underconnectivity theory of ASD (Just et al., 2004), which will be discussed in more detail later, but briefly suggests ASD results from underconnectivity at the global level while there is overconnectivity at the local level, i.e. too few or weak connections *between* regions of the brain but too many or too strong connections *within* regions of the brain. One study has found exactly this pattern in the default mode network (Washington et al., 2013) but another study looking explicitly at how the level of functional connectivity varied with distance between the regions found no relationship (Supekar et al., 2013). However, Supekar et al. (2013) also looked at the amplitude of the low frequency fluctuations (ALFFs) within cortical regions across the whole brain. ALFFs provide a measure of the regional changes in signal level and were found to be higher in ASD which the authors argue is consistent with a more subtle pattern of an altered ratio of excitation to inhibition (Supekar et al., 2013).

Although there are a growing number of studies which report normal levels of functional connectivity (Tyszka et al., 2013) or widespread increased functional connectivity (Di Martino et al., 2011; Lynch et al., 2013; Supekar et al., 2013; Uddin et al., 2013a) in ASD, there are a couple of methodological factors that need to be taken into account. Firstly, a number of these studies have been conducted in high-functioning individuals who may not be representative of ASD individuals as a whole. Secondly, as Müller et al. (2011) noted in their recent review of functional connectivity findings in ASD, those studies that were consistent with the underconnectivity theory of ASD used an ROI based approach, whereas the majority (7 out of 11) of studies that did not find reduced connectivity employed a whole-brain approach. More stringent corrections for multiple

comparisons must be employed in a whole brain approach, which may account for reduced detection of underconnectivity that is only present in some areas of the ASD brain. However, many of these studies found increased functional connectivity in ASD, which would not be expected if higher correction factors were responsible for the different results found by these different approaches (Müller et al., 2011).

Perhaps the most interesting aspect of the divergent findings is that, as Uddin et al. (2013b) note, there is a developmental aspect to be considered, with most of the studies reporting underconnectivity having been conducted in adult or adolescent samples. Given the developmental nature of autism this is an important point. Of the studies discussed here six were conducted in children aged 13 or below, four of these (Di Martino et al., 2011; Lynch et al., 2013; Supekar et al., 2013; Uddin et al., 2013a) reported overconnectivity in ASD. Of the two that did not find overconnectivity, one looked only at connectivity between voice-selective areas of cortex and other areas of the brain (Abrams et al., 2013) and so is limited in what it can say about over- or under-connectivity in other regions. The other study looked in children between 1 and 3.5 years of age and looked at interhemispheric synchronisation (Dinstein et al., 2011), which could show a different pattern of functional connectivity to that shown between different regions across the brain. In addition, this is a much younger age group than those included in studies that show overconnectivity, and so may reflect another developmental time point and different pattern of levels of connectivity.

It would seem therefore, as noted by Uddin et al. (2013b), a pattern is emerging of overconnectivity in ASD at young (pre-pubertal) ages that contrasts with the largely consistent findings of underconnectivity at post-pubertal ages. Indeed several other studies have highlighted the effect of age, finding both that there is a greater decrease in interhemispheric connectivity with age in typically developing controls than is seen in

ASD (Anderson et al., 2011) and that participants with ASD show more similar functional connectivity in the default mode network to younger (6-9 year old) than older (10-17 year old) typically developing controls (Washington et al., 2013).

Neuropathological findings

Due to the scarcity of suitable post-mortem tissue, there is much less data available on neuropathological changes in ASD than MRI differences. However, just as much variation in the findings can be seen.

One of the most consistent neuropathological findings is of decreased numbers of Purkinje cells in the cerebellum (Kemper and Bauman, 2002; Palmen et al., 2004; Ritvo et al., 1986; Skefos et al., 2014), although this has not been found in all studies (Bauman and Kemper, 1996; Fatemi et al., 2002). Reductions in cell density have also been reported in both cortical (van Kooten et al., 2008) and subcortical (Schumann and Amaral, 2006) areas, although a number of studies have also found no difference (Coleman et al., 1985; Jacot-Descombes et al., 2012; Morgan et al., 2012; Mukaetova-Ladinska et al., 2004; Oblak et al., 2011b; Santos et al., 2011; Zikopoulos and Barbas, 2010) or increased cell densities (Courchesne et al., 2011b; Kemper and Bauman, 2002).

In addition to differences in cell densities, a number of studies have reported alterations in cell size (Casanova et al., 2013; Kemper and Bauman, 2002; Raymond et al., 1995; van Kooten et al., 2008), even in the absence of cell density differences (Fatemi et al., 2002; Jacot-Descombes et al., 2012). These findings have important implications for the expected pattern of connectivity as the length of a cell's axon is proportional to the size of the cell body and so smaller neurons in ASD would bias the pattern of corticocortical connectivity towards shorter connections (Casanova et al., 2013). Interestingly, the differences in cell size seem to be age related. Jacot-Descombes et al. (2012) found

smaller neurons in patients with ASD in BA44 and BA45. This difference was most pronounced at young ages. Controls showed a negative relationship between age and pyramidal neuron volume, which was absent in patients with ASD, resulting in no difference in pyramidal neuron volume being seen between groups in adulthood. This finding is consistent with the idea of an early bias towards the establishment of more local connections at the expense of long-distance connections (Courchesne and Pierce, 2005b). However, it seems that the direction of change in cell size with age may be region specific. Larger neurons have been observed in both the nucleus of the diagonal band of Broca and the inferior olive of patients with ASD aged under 12 years, whereas ASD patients over 18 years have smaller neurons in these areas compared to controls (Kemper and Bauman, 1998; Kemper and Bauman, 2002). Although this clearly demonstrates the importance of studying cases from across the life-span, data from young cases is lacking, with the average age of cases investigated in post-mortem studies of ASD being 21 years (Courchesne et al., 2007).

In addition to changes in size and density of neurons, a number of other neuropathological changes have been reported in ASD, including quantitative measures of glial cell density increases (Morgan et al., 2010) and decreases (Mukaetova-Ladinska et al., 2004), changes in microglia-neuronal spatial clustering (Morgan et al., 2012), alterations in axonal size and density (Azmitia et al., 2011; Zikopoulos and Barbas, 2010) and increased dendritic spine density (Hutsler and Zhang, 2010); as well as qualitative reports of dysplasias (Hutsler et al., 2007; Wegiel et al., 2010), disturbances in lamination (Bailey et al., 1998; Hutsler et al., 2007; Kemper and Bauman, 1998; Mukaetova-Ladinska et al., 2004; Oblak et al., 2011b; Wegiel et al., 2010), the presence of neurons in subcortical white matter (Bailey et al., 1998; Hutsler et al., 2007; Oblak et

al., 2011b) and a less distinct grey-white matter boundary (Avino and Hutsler, 2010; Bailey et al., 1998; Hutsler et al., 2007).

As can be seen, a huge range of different abnormalities have been reported in the cortex in ASD, including some reports of no differences at all (Bauman and Kemper, 1985; Coleman et al., 1985; Kemper and Bauman, 2002; Williams et al., 1980), but with no consistent findings really emerging. However, a series of recent studies have consistently reported reductions in the width of components of the minicolumnar structure of the cortex (Buxhoeveden et al., 2006; Casanova et al., 2006a; Casanova et al., 2002b; Casanova et al., 2002c; Casanova et al., 2010; Casanova et al., 2006b). Minicolumns form the fundamental structural unit of the cortex, and as the neurons within an individual minicolumn respond to slightly different stimuli to the neurons in a neighbouring minicolumn, they also form a physiologically defined unit (DeFelipe, 2005; Mountcastle, 1997; Peters, 2010). Therefore, as will be discussed later, alterations in minicolumnar organisation could have important implications for the structure and functioning of the cortex.

Psychological Theories of ASD

ASD as a deficit in Theory of Mind

One of the most popular psychological explanations of ASD is that it stems from a lack of, or delay in development of, a 'theory of mind'. The concept of a 'theory of mind' (ToM) was first explored by Premack and Woodruff (1978) who defined it as the ability to attribute mental states, both to oneself and others. These mental states include thoughts, beliefs and desires and an important aspect of ToM is the ability to appreciate that the mental states of others may differ from one's own, and that they may not be true.

In typical development, ToM usually develops by 4-6 years of age (Wimmer and Perner, 1983) and has been proposed to build on the ability to construct metarepresentations, such as 'Sally believes the chocolate is in the cupboard', initially learned through pretend play, something that is less common in ASD (Leslie, 1987). ToM has been suggested to be necessary in many aspects of social interaction, such as understanding the actions of others, and communication as it is necessary to take account of the background knowledge of other participants in the conversation (Baron-Cohen, 1990).

Given that ASDs often feature lower levels of pretend play, difficulties in social interaction and communication, particularly the reciprocal aspects such as turn taking; it has been suggested that there may be a deficit in development of ToM (Baron-Cohen et al., 1985). A commonly used task in the investigation of ToM is the false belief task and in particular the Sally-Anne task. This involves introducing the child to two characters, Sally and Anne, one of whom, Sally, places a marble in a basket, before leaving the room to go for a walk. While she is gone, Anne takes the marble out of the basket and places it in a box. When Sally returns the children are asked where Sally will look for the marble. This requires the children to understand that Sally has a false belief that is different to their own belief about where the marble is. Baron-Cohen et al. (1985) found that while 85% of typically developing children and 86% of children with Down's syndrome were able to correctly perform the task, 80% of children with ASD *failed* the task. Inclusion of the group of children with Down's syndrome allowed mental retardation to be ruled out as the reason for the difficulties of most of the ASD group with the task. However, children with ASD are also found to have difficulties with an easier version of this task, the windows task (Hughes and Russel, 1993). Here there are two boxes, each with a window facing the child. One of the boxes contains a chocolate (only the child can see which one), and the children are required to tell the experimenter which box to look in. If

the experimenter find the chocolate, they keep it, if they don't the child gets to keep it. Children with ASD were found to have difficulties pointing to the box without the chocolate, even when the examiner was removed. This task did not necessarily require the use of ToM, as they should have been able to learn the relationship between pointing to the empty box and receiving the reward suggesting that they were unable to disengage from the object and inhibit the more salient response, a failure of executive function (Hughes and Russel, 1993).

Another variation on the false belief task that has been useful in investigating ToM is the false photograph task. In this task children were shown a doll, Judy, who is initially wearing a red dress, and take a photograph of the scene. While the photo is developing Judy is changed into a green dress and the children are asked what colour dress she is wearing in the photo (without looking). A false belief task of the same scenario is also conducted, only with another doll, Susan, present instead of taking a photo, with children being asked what dress will Susan expect to see Judy wearing when she returns (Leekam and Perner, 1991). Typically developing children struggle equally with the false belief task and the false photograph task until around age 4 when they become able to pass both, whereas children with ASD are able to pass the false photograph task when they cannot pass the false belief task (Leekam and Perner, 1991; Leslie and Thaiss, 1992). Although Leekam and Perner (1991) have argued that this goes against Leslie (1987) claim that there is a metarepresentational deficit in ASD, other studies have claimed that ability to pass the false photograph task supports a metarepresentational deficit relating to mental states only (Charman and Baron-Cohen, 1992; Leslie and Thaiss, 1992).

While a theory of a deficit in ToM would seem to concentrate on the social and communication aspects of ASD, it has been argued that as such a deficit renders the world unpredictable, restricted and repetitive behaviours emerge as a defence mechanism

to cope with this (Baron-Cohen, 1996; Pellicano, 2011), this does not stand up against the fact that restricted and repetitive behaviours are seen across the spectrum (Pellicano, 2011). In addition, this theory has been criticised as it relies on failure on false belief tasks indicating lack of theory of mind, while it has been argued that failure on such tasks could be due to problems with executive function or even language (Pellicano, 2011).

Although a deficit in ToM might seem an appealing theory due to its ability to explain social and communication abnormalities in ASD, it is unable to explain some of the non-social aspects of ASD, in particular aspects such as islets of ability and preoccupation with parts of objects and arguably restricted and repetitive behaviours (Frith and Happé, 1994).

ASD as an Extreme Male Brain

A related theory, attempting to account for the spectrum of conditions seen in ASD, is the extreme male brain (EMB) theory (Baron-Cohen, 2003). This works from the premise that ‘The female brain is predominantly hard-wired for empathy. The male brain is predominantly hard-wired for understanding and building systems.’ (Baron-Cohen, 2003). In this view females tend to be better at what is called empathising (recognition of others’ emotions accompanied by an appropriate emotional reaction on their own part), whereas males are better at systemising (finding out how systems work or understanding things in terms of parts that function as a system) (Baron-Cohen, 2003). As individuals with ASD are characterised by poor empathising, as evidenced through social impairments, but also high systemising, as can sometimes be seen through restricted and repetitive behaviours or savant abilities, the EMB theory of ASD suggests that ASD is due to the presence of this extreme male pattern of thinking (high systemising and low empathising)(Baron-Cohen, 2003).

As can be seen, this theory still puts emphasis on the theory of mind component, which would be expected to underlie empathising, but builds on the idea of a deficit in ToM, by evoking the concept of systemising, both to account for strengths in ASD and to provide a more robust account of the presence of restricted and repetitive behaviours.

Although this theory is well supported in terms of data finding higher scores on tests of systemising, and lower scores on tests of empathising in ASD (Baron-Cohen et al., 1997a; Baron-Cohen et al., 2003; Baron-Cohen et al., 2001; Baron-Cohen et al., 1997b; Lawson et al., 2004), for a theory based on characterising gender differences in the general population, this theory neglects a lot of other information on gender differences in ASD. In particular, this theory does not take into account the issues discussed above relating to a potential bias in diagnosis rates (Kreiser and White, 2013), differences in presentation (Kreiser and White, 2013; Rutter et al., 2003), or the fact that the diagnostic criteria and questionnaire measures of ASD may be more representative of males than females (Williams et al., 2008). If the previously accepted gender ratio of 4:1 is not in fact correct, and those identified as having ASD using traditional assessment methods are not representative of the ASD population as a whole, then this calls into question the very basis on which the EMB theory is founded.

Weak Central Coherence

In contrast to previous theories which focussed on explaining the deficits in ASD, weak central coherence was proposed as an explanation of the areas of preserved, or even enhanced, skills. For example, it was found that participants with ASD showed better performance than controls on the Embedded Figures Task (Shah and Frith, 1983). This task requires participants to identify the figure (e.g. a geometric shape) within a larger, meaningful picture, as quickly as possible. Therefore Frith (2003) suggested that

typically developing controls process information as an integrated whole (i.e. globally) in order to extract meaning, for example in remembering the meaning of a sentence rather than the exact wording. This tendency to process the information together was termed 'central coherence'. In contrast, it was suggested that individuals with ASD show 'weak central coherence', that is they process stimuli in terms of their features rather than as a whole (Frith, 2003). Although the original theory suggested a deficit in central coherence, findings of preserved performance on tasks requiring global processing meant that later revisions emphasised a *bias* towards a more local processing style, rather than this being the only processing style used (Happé and Frith, 2006).

Although the original theory suggested that a local processing bias was the primary cause of autism, able to account for both the social and non-social aspects, it was later found that performance on tasks of central coherence did not correlate with theory of mind ability, suggesting that the social aspects were not caused by the local processing bias (Happé and Frith, 2006; Happé, 1997). Instead, the focus has been shifted to a model where several primary cognitive abnormalities co-occur and together lead to ASD, with weak central coherence being associated primarily with non-social aspects (Happé et al., 2006; Pellicano, 2011).

ASD as a disorder of perception

Building on Lavie's load theory of attention and cognitive control (Lavie, 2005), which suggests that irrelevant distractors are processed only when the task being performed does not exhaust the perceptual capacity, Remington et al. (2012) have suggested that this might be a useful framework in which to consider ASD. They suggest that previous studies of attention and perception in ASD are broadly split between showing superior visual attention on one hand, and on the other greater distractibility and processing of

irrelevant information. This is argued, therefore to be consistent with the view that there is in fact a higher perceptual capacity in ASD, such that on tasks where control subjects' perceptual capacity is 'full', resulting in processing of just the stimuli and not distractors, subjects with ASD still have spare perceptual capacity and so process the distractors. Equally, this enhanced perceptual capacity is able to allow them to show enhanced performance compared to controls at high perceptual loads (Remington et al., 2012). This theory is in some ways similar to the theory of enhanced perceptual functioning (Mottron et al., 2006), which suggests that in ASD perceptual processes are superior to higher-order processes, in that it stresses the importance of lower-level processes. However, an important difference in the theory of Remington and colleagues is that it does not necessarily suggest any difference in the perceptual processes themselves, rather in the capacity for perception.

This theory of enhanced perceptual capacity has been supported by a number of recent studies which find that high levels of perceptual load reduce processing of distractors in controls, but not in subjects with ASD (Adams and Jarrold, 2012; Remington et al., 2009; Remington et al., 2012) (although one study reported no difference in perceptual capacity between ASD and control subjects (Ohta et al., 2012)). In fact perceptual capacity has been demonstrated to vary with Autism Spectrum Quotient scores (a measure of non-clinical ASD traits) in the general population (Bayliss and Kritikos, 2011).

Although this theory seems to provide a resolution between what seemed like two incompatible bodies of work, showing both increased perceptual abilities and increased distractibility in ASD, it does not seem able to account for all aspects of ASD. For example it seems difficult to reconcile such enhanced perceptual capacity with the intense restricted and repetitive behaviours seen in ASD.

Biological Theories of ASD

ASD as a disorder of altered developmental trajectory

Based on findings from a number of structural MRI scans (Carper et al., 2002; Courchesne et al., 2001; Redcay and Courchesne, 2005), Courchesne and colleagues have suggested that there is an altered developmental trajectory in autism (Courchesne and Pierce, 2005a). This suggests that, although in ASD brain volume is normal, or even reduced, at birth, there is a period of over growth at early ages, resulting in increased brain volume compared to controls, followed by a period of arrested growth and possibly even decline in brain volume at older ages (Courchesne et al., 2011a). This period of overgrowth has been noted to coincide with the onset of autistic symptoms (Courchesne et al., 2011a).

In addition, the degree of overgrowth is not constant across the whole brain, and has been found to be more pronounced in frontal regions (Carper et al., 2002; Herbert et al., 2004), and it has been suggested that it is those parts of the brain that have more protracted development that are the most affected (Courchesne et al., 2011a; Herbert et al., 2004). While this theory arguably does little more than describe the data, it provides an important framework for interpreting the findings of other studies in terms of altered developmental trajectories, particularly one where development is accelerated in early childhood. Such a model of disrupted developmental timing is able to explain other neurological findings or models, such as the idea of disrupted cortical connectivity. For example, early brain overgrowth might influence the relative costs of wiring between different areas (it is known that the relative size of the corpus callosum is reduced with increasing forebrain size (Jancke et al., 1999; Jäncke et al., 1997) causing a decrease in communication between the hemispheres and an increase in hemispheric specialisation)

and also the pattern of connections that develop (Lewis and Elman (2008) developed a computational model that demonstrated that brain overgrowth of the sort seen in ASD leads to changes in connectivity favouring more local connections).

ASD as a disorder of connectivity

In their ‘underconnectivity theory’ Just et al. (2004) suggest that ASD is caused by ‘underfunctioning of integrative circuitry’ i.e. those long-range connections responsible for emergent properties of the brain resulting from integration of information from different areas of the brain. Therefore it is suggested that deficits will be observed on tasks requiring higher level, integrative processes. This theory is similar, therefore, to the weak central coherence theory (Happé and Frith, 2006) in that preserved performance is expected on tasks not requiring integration between different areas of the brain. The underconnectivity theory as conceptualised by Just et al. (2004) is concerned primarily with functional connectivity, and makes no comment on physical connectivity of the brain. However, this theory has been popular in providing an explanation of how some of the structural findings could relate to ASD symptomatology, particularly in light of extensions of the theory which suggest that long-distance underconnectivity is complemented by local overconnectivity (Courchesne and Pierce, 2005b; Kana et al., 2011; Wass, 2011).

This theory is supported not only by evidence of abnormalities in connectivity (as described above), but also structural findings. For example, Herbert et al. (2004) found that white matter enlargement was restricted to the radiate compartment, that is the compartment immediately underlying the cortex and consisting predominantly of short, and medium, range fibres (Zikopoulos and Barbas, 2013). Zikopoulos and Barbas (2010) found that in ASD subjects the white matter underlying the anterior cingulate cortex

contained a reduced density of the large axons which mediate long range connections, but an increased density of the small axons which mediate short range connections, when compared to controls. However, a similar difference in the size of axons was not seen for either the prefrontal cortex or orbital frontal cortex, suggesting that perhaps the pattern of long-range overconnectivity and short-range underconnectivity is not uniform across all brain regions. Indeed, Courchesne and Pierce (2005b) have put forward the idea that it is the frontal cortex which shows excessive intra-areal connectivity, but reduced connectivity with other regions of the brain. A recent study by Ecker et al. (2013) investigated the connectivity within the grey matter itself, finding reduced local wiring costs, particularly in fronto-temporal areas, consistent with local overconnectivity. Furthermore, the reduced wiring costs were found to correlate with severity of social and repetitive symptoms (Ecker et al., 2013), providing evidence that such changes in patterns of connectivity may relate directly to ASD symptomatology.

ASD as a disorder of an altered excitation/inhibition ratio

Based on evidence of a high co-morbidity of epilepsy in ASD (Gillberg and Billstedt, 2000) and high rates of epileptiform and unusual EEG activity in ASD (Orekhova et al., 2007) (Lewine et al. (1999) found epileptiform activity in 82% of children with ASD), Rubenstein and Merzenich (2003) have suggested that a common underlying explanation of ASD may be the presence of noisy cortical networks caused by an increased ratio of excitation to inhibition. This altered ratio could be caused by alterations in either the glutamatergic or GABAergic systems, the latter of which is supported by evidence from genetics studies of common variants (Persico and Napolioni, 2013), findings of reduced numbers of GABA receptors (Oblak et al., 2010; Oblak et al., 2011a), reduction in the number of inhibitory interneurons in prefrontal cortex (Zikopoulos and Barbas, 2013)

(although this has not been replicated in other areas of cortex (Oblak et al., 2011b)), as well as findings of reduction in the neuropil component of cortical minicolumns, the location of the GABAergic interneurons (Casanova, 2006; Casanova et al., 2006b).

There is also support for a model of altered excitation and inhibition from behavioural tasks. Subjects with ASD have been found to show impaired performance on somatosensory tactile spatial localisation capacity tasks, something that is consistent with reduced cortical inhibition (Tannan et al., 2008; Tommerdahl et al., 2007; Tommerdahl et al., 2008). Using a computational model, Vattikuti and Chow (2010) show that increased excitation in a system is sufficient to cause the saccade abnormalities seen in ASD.

However, computational models of the development of minicolumns and cortical feature maps suggest that decreased levels of inhibition would lead to the development of wider minicolumns (Buxhoeveden, 2012; Gustafsson, 2004), which is the opposite to what has previously been found in ASD (Buxhoeveden et al., 2006; Casanova et al., 2006a; Casanova et al., 2002b; Casanova et al., 2002c; Casanova et al., 2010). Despite this, wider minicolumns (as would be predicted from reduced inhibition in ASD) have been suggested to facilitate greater discrimination of stimuli (rather than the holistic processing facilitated by narrower minicolumns) (Chance et al., 2012; Harasty et al., 2003), a well established feature of ASD, perhaps best captured by the weak central coherence theory (Happé and Frith, 2006).

As Dinstein et al. (2011) point out, such an imbalance could lead to random fluctuations in activity of cortical neurons (i.e. greater noise), which has been suggested to contribute to overreactivity of sensory systems (due to difficulties in distinguishing a stimulus from noise) (Liss et al., 2006), which in turn requires the recruitment of higher processing mechanisms to try to suppress this irrelevant information, resulting in more diffuse

activation of neural resources during specific tasks (Groen et al., 2008). Increased noise in cortical systems could also have developmental implications, resulting in disrupted neural representations and impairing the development of further activity-dependent relationships (Belmonte and Bourgeron, 2006).

ASD as a minicolumnopathy

Recent findings of consistently reduced minicolumnar widths in ASD (Buxhoeveden et al., 2006; Casanova et al., 2006a; Casanova et al., 2002b; Casanova et al., 2002c; Casanova et al., 2010) have led to the conceptualisation of ASD as a disorder of minicolumns (Casanova, 2006). When an imbalance of excitation to inhibition is considered the primary cause for ASD, the expected outcome would be wider minicolumns, rather than the narrower minicolumns that have been observed. However, it can also be argued that the narrower minicolumns are the primary factor, and that an imbalance in excitation and inhibition follows from a reduction in inhibitory control which results from narrowing of the inhibitory interneuron containing neuropil space (Casanova, 2006). Not only can this approach, therefore, account for all the features of ASD that would be expected to arise from an inhibition-excitation imbalance, but it is also supported by one of the first consistent neuropathological findings in ASD. In addition, narrower minicolumns in a normal, or above normal, sized brain, would result in a massive increase in the total number of minicolumns, and therefore the number of fibres needing to connect them. As these would be primarily short range fibres, this theory is also able to account for the fact that the volume increases are more pronounced in the radiate white matter (Casanova, 2006).

Buxhoeveden et al. (2006) discovered that while reductions in minicolumn width were present across a number of regions of prefrontal cortex, minicolumn width in primary

visual cortex was unaffected. From this it was suggested that only areas responsible for ‘higher-order’ processing were affected in ASD, while those responsible for basic functions, such as primary sensory cortices, were unaffected (Buxhoeveden et al., 2006). This may be able to account for the pattern of cognitive abilities seen in ASD, with deficits becoming apparent on tasks requiring ‘higher-order’, integrative processing (Happé and Frith, 2006). In addition, the more pronounced pathology in ‘higher-order’ areas, of which prefrontal cortex is a prime example, accounts for the fact that the greatest increase in white matter volume is seen there (Herbert et al., 2004). If the minicolumns are most reduced in width in these regions, then it makes sense that these are the regions which will need the most extra local connections to be formed, and so will show the greatest increase in radiate white matter volume.

Overview of the Thesis

The present work aims to test Buxhoeveden and colleague’s suggestion (Buxhoeveden et al., 2006) by explicitly contrast primary sensory regions with higher order association regions. Three key regions of interest will be studied throughout this thesis, orbital frontal cortex, primary auditory cortex and part of the inferior parietal lobe, as they are all implicated in processes affected in autism.

The work reviewed above consistently highlights the importance of taking age into account, therefore this work will provide one of the first investigations into the effect of developmental trajectory on both regional volumetric measures and cortical microstructure. Volumes of the cortical regions of interest will first be examined (Chapter 2), as this could have important implications for the interpretation of any differences in cortical microstructure (for example, increased regional volume in the presence of ‘typical’ or reduced minicolumn width would suggest greater numbers of

minicolumns within a region). This thesis will then go on to investigate cortical organisation by characterising minicolumnar width in these regions (Chapter 3). This will form an important contribution to the literature as it will not only explicitly test whether primary sensory regions are unaffected in ASD, but will also investigate the developmental trajectory of any diagnostic differences in minicolumn width, and provide the first investigation of minicolumnar organisation in several regions in ASD (inferior parietal lobe and primary auditory cortex).

The final part of this thesis forms the first quantitative study of the relationship between measures of cortical diffusion and the microstructure of the cortex (Chapters 4, and 5). Given the diagnostic differences in cortical organisation that have been previously shown, this method could form the basis of a powerful way of potentially detecting these changes in-vivo.

Chapter 2:

In-vivo Volumetric Investigation

Introduction

Whole-brain Volumetric Differences

Enlarged brain volume is one of the most consistent findings in ASD (Stanfield et al., 2008). Meta-analyses of data across the life span suggest ASD is characterised by smaller brain size at birth, compared to typically developing children, followed by a period of intense overgrowth during the first year (Redcay and Courchesne, 2005). This accelerated growth is then thought to plateau, such that by adolescence brain size is only marginally larger in ASD (Redcay and Courchesne, 2005) and in fact may begin to decline, leading to reduced brain volume compared to controls in later adulthood (Courchesne et al., 2011). For very young children (<2 years) it is often not possible to acquire MRI scans and so brain size is assessed by looking at head circumference, which is a very good proxy for brain size (Raznahan et al., 2013). Many of these studies, however, compared measures from ASD to established population norms, which recent studies have suggested do not accurately characterise the population (Daymont et al., 2010; Raznahan et al., 2013). Raznahan et al. (2013) have found that studies that compare head circumference in ASD infants with a locally recruited control group rather than CDC and WHO population norms are less likely to find evidence of brain overgrowth in the first year. In fact, they found that if typically developing controls are compared to these norms, they show a similar pattern of ‘overgrowth’ in the first year to that seen in infants with ASD. However, Raznahan et al. (2013) did find evidence for

slightly larger brains in ASD by two years of age, suggesting that these differences are smaller and start later than previously thought.

Although studies find brain overgrowth between about two and five or six years on average in ASD (Courchesne et al., 2011; Raznahan et al., 2013), it is also clear that there is considerable variation within this (Suren et al., 2013), with individuals with ASD showing greater rates of both macrocephaly and microcephaly (Ben-Itzhak et al., 2013); although this was based on head circumference measures compared to population norms and so may over-estimate the rate of macrocephaly. Interestingly there seemed to be an effect of gender, with females with ASD showing lower mean head circumference compared to typically developing females, and greater rates of microcephaly (Ben-Itzhak et al., 2013; Suren et al., 2013). Females in these studies, however, also showed greater rates of developmental regression, genetic disorders, epilepsy or intellectual disability. This highlights the potentially confounding nature of comorbidities and the severity of the impairment.

While this heterogeneity should not be overlooked, there still seems to be a trend towards increased brain volume at young ages in ASD which is worth investigating. In a study of children between 2 and 11 years only the youngest group (2-4 years) differed from controls, with differences restricted to the frontal and parietal lobes for white matter, and frontal and temporal lobes for grey matter (Carper et al., 2002). As would be expected from findings of early overgrowth, while typically developing controls showed an increase in white matter volume of 45% in frontal and parietal lobes between ages 2 and 11, this was attenuated in ASD with white matter volume increasing by just 13%. Similarly in grey matter, while controls showed an increase in volume of 20% in the frontal lobe between 2 and 8, in ASD this was only 1% (Carper et al., 2002). Further investigation of the location of white matter increases in ASD compared to controls

revealed them to be primarily in the radiate white matter, that is the corona radiata and the short association fibres directly beneath the cortex. The areas showing greater volume increases compared to controls were those areas that myelinated later or for longer (Herbert et al., 2004). These findings are consistent both with a post-natal onset of brain overgrowth as well as the theory that there is greater local connectivity in ASD when compared to controls, as short association fibres are responsible for connections between neighbouring regions of cortex.

Localised Volumetric Differences

A number of grey and white matter structures have been identified as showing altered volume in ASD on the basis of region of interest (ROI) analyses, with some areas showing both increases and decreases (Brambilla et al., 2003). Some of the more consistent findings have been of reduced volume of the corpus callosum and increased volume of the caudate and amygdala (Brambilla et al., 2003; Stanfield et al., 2008). Again, findings relating to the amygdala have highlighted the importance of taking age into account with larger amygdalae being seen in ASD during childhood (Schumann et al., 2009) but not adolescence (Schumann et al., 2004). However, a recent longitudinal study found no difference in amygdala volumes even at comparably young ages (Barnea-Goraly et al., 2014). It should be noted that although this was a longitudinal study participants were only followed up after two years and so none covered the full age range being investigated (8-14 years) and also that the number of participants completing both scans was relatively small (9 ASD and 14 controls). Although it is still not entirely clear when or whether the amygdala is enlarged, some interesting correlations with clinical features have been found. Amygdala enlargement has been found to be greater in ASD than Asperger's (Schumann et al., 2004), larger amygdala volume at 3 years was found to

relate to poorer social and communication skills (Munson et al., 2006; Schumann et al., 2009) and appropriate use of eye-contact was related to amygdala volume increases between 8 and 14 (Barnea-Goraly et al., 2014), implicating the amygdala in social and communication aspects of ASD. Similarly, caudate volume has been shown to correlate with ADI scores of restricted and repetitive behaviours (Hollander et al., 2005; Sears et al., 1999).

Voxel-based morphometry (VBM) is an unbiased whole brain approach to investigation of structural differences, requiring no a priori hypotheses about where these differences may be located. Structural MRI scans are first registered to a template (created by averaging scans from the subjects included in the study). This registration is not perfect or it would remove the localised structural differences of interest, but the aim is to remove large scale differences in structure and position that would otherwise affect the results. The registered images are then segmented into grey and white matter before being smoothed to create an image where each voxel contains an average grey matter density calculated over the surrounding voxels. The smoothing step also creates more normally distributed data. These smoothed images can then be compared on a voxel by voxel basis to look for localised differences in grey matter density (Ashburner and Friston, 2000). Alternatively the images can be 'modulated' in order to enable investigation of volumetric differences. In order to modulate the spatially normalised tissue is multiplied by the amount the volume is changed by, e.g. if a region doubles in volume then modulation will account for this by halving the intensity of the region. This ensures all the original information about the volume of this region is retained and so comparisons of volume can be made. There are some limitations on interpreting the findings, however, as significant differences may be due to true changes in tissue density, for example through cortical thinning, but equally may instead reflect problems in the

registration, differences in scan intensity or differences in gyration pattern between groups (Ashburner and Friston, 2001), although the last may be of interest in its own right.

A number of VBM studies have been conducted in ASD, looking at localised changes in both grey and white matter, finding differences in a range of areas (grey matter findings are summarised in Table 1). Some of the most consistently implicated regions include the cerebellum, prefrontal cortex and medial temporal lobe, including the amygdala, although the direction of change is not always consistent between studies. The studies summarised below, however, include subjects with both autism and Asperger syndrome, and subjects across a range of ages and different sexes.

There is evidence that those with Asperger syndrome show fewer structural differences compared to controls than do those with autism (McAlonan et al., 2008; Yu et al., 2011). In addition, previous studies have found different patterns of structural differences in Asperger syndrome and autism (Yu et al., 2011). Therefore, considering all these studies together may be giving the impression of more disagreement than actually exists. Indeed, if studies for which the sample included only subjects with Asperger syndrome or high-functioning autism are considered, a more consistent pattern begins to emerge with a number of studies finding decreases in grey matter density in the hippocampus and increases in the cerebellum, fusiform gyrus and inferior parietal lobe, particularly BA40 (Abell et al., 1999; Brieber et al., 2007; Ke et al., 2008; McAlonan et al., 2002; McAlonan et al., 2008; Salmond et al., 2005; Yu et al., 2011). However, these results are still not entirely consistent with some studies finding no effect or opposite effects (Kosaka et al., 2010; McAlonan et al., 2008; Salmond et al., 2005; Yu et al., 2011). Also, some of the studies summarised below do not provide information on the composition of

their sample and so may have been incorrectly excluded from the Asperger syndrome and high functioning autism group.

Another factor that varies between studies, and is known from ROI-based studies to have a different effect on structure in ASD and control subjects, is age. While a few VBM studies have looked in childhood and early adolescence, most have included large age ranges from mid-adolescence through adulthood. Looking at those studies that look only at children below the age of 15 a more consistent pattern of increased grey matter density in inferior parietal lobe, particularly BA40, and decreases in prefrontal and orbital frontal cortices, basal ganglia structures, superior temporal gyrus and medial temporal lobe, with almost all findings going in the same direction, although not all studies showed differences in the same areas (Brieber et al., 2007; Jiao et al., 2010; Ke et al., 2008; McAlonan et al., 2005; McAlonan et al., 2008; Mengotti et al., 2011). In contrast, although findings are more mixed in samples including adults, most studies seem to find increased grey matter density and this seems to be particularly consistent in the cerebellum, fusiform and caudate (Abell et al., 1999; Bonilha et al., 2008; Cauda et al., 2011; McAlonan et al., 2002; Nickl-Jockschat et al., 2012; Rojas et al., 2006; Salmond et al., 2005; Salmond et al., 2007; Waiter et al., 2004; Yu et al., 2011) although see also (Craig et al., 2007; Rojas et al., 2006; Toal et al., 2010). In addition a number of VBM studies have investigated correlations between age and local grey matter density finding, among others, a negative correlation between age and right supramarginal gyrus (Rojas et al., 2006), and caudate volume in controls but not ASD (McAlonan et al., 2002), and while the same negative quadratic pattern of amygdala volume change with age was observed in control and ASD subjects, this curve was shifted to the left in ASD subjects (Greimel et al., 2013). These studies clearly demonstrate the importance of taking age into account as this could affect the location and direction of any changes found.

| Study | PFC | OFC | PC | C | MC | SC | I | BG | STG/ STS | MTG | ITC | MTL | TPJ | SPL | IPL | OL | Cb | Tm | Bs |
|--------------------------------|-----|-----|----|---|----|----|-----|-----|-------------|-----|-----|-----|-----|-----|-----|----|----|----|----|
| (Riva et al., 2011) | * | | | | * | * | * | * | * | * | * | | | * | * | * | * | | |
| (Nickl-Jockschat et al., 2012) | | | | | | | | ↓ | | | | ↓ | | | | ↑ | | | |
| (Toal et al., 2010) | | | | | | | | | ↓ | | ↓ | ↓ | | | | | ↓ | | |
| (Cauda et al., 2011) | | | | ↑ | ↓ | | ↓ ↑ | ↓ ↑ | | ↑ | ↑ | ↓ | | ↓ ↑ | ↓ | ↑ | ↑ | | |
| (Rojas et al., 2006) | ↑ | | | | ↑ | ↑ | | ↑ | | ↑ | ↑ | ↑ | | | | | ↓ | | |
| (Calderoni et al., 2012) | ↑ | | | | | | | | | | | | * | | | | | | |
| (Hyde et al., 2010) | ↑ | ↑ | | | ↓ | ↓ | | | | | | | | | | | | | ↑ |
| (Boddaert et al., 2004) | | | | | | | | | ↓ | | | | | | | | | | |
| (Ke et al., 2008) | ↑ | | | | | ↑ | | | | | | ↓ | | | ↑ | | ↑ | | |
| (Kosaka et al., 2010) | ↓ | | | | | | ↓ | | | | | | | | ↓ | | | | |
| (Jiao et al., 2010) | ↓ | ↓ | | ↑ | | | | | | | | ↓ | | | | | | | |
| (Mueller et al., 2013) | ↓ | | ↓ | | | | | | | ↓ | ↓ | | ↓ | | | | | | |
| (Waiter et al., 2004) | ↑ | | | ↑ | | | | | ↑ | ↑ | ↑ | ↑ | | | | ↑ | | | ↓ |
| (McAlonan et al., 2002) | ↑ | | | ↑ | | | | ↑ | | | | | | | | | ↑ | ↑ | |
| (McAlonan et al., 2008) | ↓ | | | ↓ | | ↓ | ↓ | ↓ | ↓ | | | ↓ | | | ↓ | | ↓ | ↓ | |
| (McAlonan et al., 2005) | ↓ | ↓ | | ↓ | | | | ↓ | ↓ | | ↓ | ↓ | | ↓ | | | | | ↓ |

| Study | PFC | OFC | PC | C | MC | SC | I | BG | STG/STS | MTG | ITC | MTL | TPJ | SPL | IPL | OL | Cb | Tm | Bs |
|------------------------|-----|-----|----|---|----|----|---|----|---------|-----|-----|-----|-----|-----|-----|----|----|----|----|
| (Brieber et al., 2007) | | | | | ↓ | ↑ | | | | | ↓ | ↓ | | | ↑ | | | | |
| (Abell et al., 1999) | ↓ ↑ | | ↓ | | | | | | | ↑ | | ↑ | | | | ↓ | ↑ | | |
| (Kwon et al., 2004) | | | | ↓ | | | | | | ↓ | ↓ | | ↓ | | | | | | |
| (Salmond et al., 2005) | ↑ | | | | | | | | | | ↓ ↑ | ↑ | | | | ↑ | ↑ | | |
| (Craig et al., 2007) | ↓ | ↓ | | ↓ | | | | ↓ | | | | ↓ | | | | ↓ | | | |
| (Greimel et al., 2013) | | | | ↓ | | | | | ↓ | ↓ | | | | | | | | | |
| (Salmond et al., 2007) | ↓ | | | | ↓ | | ↓ | | | | ↑ | | | | | | ↑ | | |
| (Schmitz et al., 2006) | ↑ | | | ↑ | | | | | | | | | | | | | | | |
| (Bonilha et al., 2008) | ↑ | | | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |

Table 2.1. Summary of grey matter differences found in VBM studies of ASD. ↑ indicates increased in ASD; ↓ indicates reduced in ASD; * indicates direction of difference was not reported. PFC=prefrontal cortex; OFC=orbital frontal cortex; PC=paracingulate; C=cingulate; MC=motor cortex; SC=somatosensory cortex; I=insula; BG=basal ganglia; TP=temporal pole; STG/STS=superior temporal gyrus and superior temporal sulcus; MTG=middle temporal gyrus; ITC=inferior temporal cortex; MTL=medial temporal lobe; TPJ=temporo-parietal junction; SPL=superior parietal lobe; IPL=inferior parietal lobe; PP=posterior parietal; PO=parieto-occipital; OL=occipital lobe; Cb=cerebellum, Tm=thalamus; Bs=brain stem.

Given the huge variation in structural findings that have previously been reported (Table 2.1), this study focuses on a more hypothesis driven approach, investigating volumetric differences in regions implicated in process which have been shown to be altered in ASD. In addition, given the suggestion discussed above, that subjects with Asperger's and high functioning autism may show a different pattern of structural differences to those with low functioning autism, the present study will focus on a more homogeneous group of high functioning subjects.

Abnormal Timing in ASD

It has been suggested that a deficit in timing ability may impair processing of social stimuli and so give rise to some of the core symptomatology of ASD (Welsh et al., 2005). Although a couple of studies have found no difference in interval timing, the ability to estimate durations, (Jones et al., 2009; Mostofsky et al., 2000) other studies have shown impairment on this task in ASD (Falter et al., 2012b; Martin et al., 2010; Szelag et al., 2004). In addition, several studies have suggested that when asked to reproduce intervals between 1-5 seconds ASD participants tend to produce intervals that overestimate short intervals and underestimate the long intervals producing responses around 3 seconds (Martin et al., 2010; Szelag et al., 2004).

In contrast, the ability to detect the time of onset of events has been much less studied in ASD. It has been shown that presentation of an auditory stimulus alongside the visual stimuli to be detected alters performance. This has been found to occur over a wider range of temporal intervals between the visual and auditory stimulus in ASD compared to controls, which has been argued to provide evidence for the ability to integrate multisensory information over a wider temporal window (Foss-Feig et al., 2010; Kwakye et al., 2011). In contrast, other studies have suggested quite the opposite, finding increased temporal resolution in ASD implying reduced integration between time points

(Falter et al., 2012a; Nakano et al., 2009). Participants with ASD have also been found to not show the impairment in ability to make temporal order judgements about tactile stimuli seen in controls in the presence of a synchronised conditioning stimulus. This has been interpreted to indicate less integration across time points and was suggested to relate to differences observed at the level of the minicolumnar structure of the cortex (Tommerdahl et al., 2008).

Neural Basis of Timing

Investigations of the neural basis of timing behaviour have largely concentrated on interval timing, using tasks that require participants to reproduce or judge the duration of an interval. Such studies have strongly implicated the basal ganglia, parietal cortex, areas of frontal cortex and the cerebellum (Grondin, 2010; Meck et al., 2008). Within the basal ganglia, most studies find evidence for the involvement of the caudate or putamen or both (Grondin, 2010; Hinton and Meck, 2004; Meck, 2006; Meck et al., 2008). Inferior parietal areas have been shown to be a functionally important part of the parietal lobe in timing behaviour, with studies implicating both the angular gyrus (BA39) (Alexander et al., 2005; Bueti et al., 2008; Harrington et al., 1998; Meck et al., 2008) and the supramarginal gyrus (BA40) (Harrington et al., 2004; Harrington et al., 1998; Meck et al., 2008; Rao et al., 2001).

There is evidence that the timing of sub-second and supra-second intervals may be supported by different patterns of neural activity, with the caudate more implicated in sub-second timing and putamen and parietal cortex more implicated in supra-second timing (Grondin, 2010; Jahanshahi et al., 2006; Meck et al., 2008). Analysis of the time course of activity during different phases of temporal processing finds activity in caudate and putamen, prefrontal and inferior parietal areas during encoding of the time interval

whereas judgements of whether the interval is shorter or longer than a standard interval activates prefrontal areas, superior temporal cortex, superior and inferior parietal cortex, putamen and nucleus accumbens (Harrington et al., 2004; Rao et al., 2001).

In contrast to interval timing which requires judgements of duration, event timing looks at what degree of temporal separation is required to judge two events as asynchronous. This can be assessed using temporal order judgements, where the order of events must be determined, and stimulus onset asynchrony tasks, where the participant must judge whether the stimuli were presented at the same or different times. Studies of event timing consistently implicate areas of temporoparietal cortex (Kanabus et al., 2002; Lewandowska et al., 2010; van Steinbuchel et al., 1999), with growing evidence for a role of right inferior parietal cortex (including the supramarginal gyrus) in tasks using visual stimuli and left inferior parietal cortex in tasks using auditory and language stimuli (Moser et al., 2009; Roberts et al., 2012; Woo et al., 2009). It has been suggested, however, that inferior parietal cortex is important for these tasks due to its role in attention rather than timing per se (Lewandowska et al., 2010; Woo et al., 2009).

The present study therefore aimed to investigate volumetric differences in ASD and how this relates to timing ability. A region of interest (ROI) based approach will be used to investigate areas previously found to show volumetric differences in ASD (amygdala and basal ganglia), areas implicated in timing behaviour (inferior parietal lobe, particularly BA40, and basal ganglia, particularly caudate and putamen) and two other cortical regions implicated in processes known to be altered in ASD; BA11 and BA41. BA11 is part of the orbital frontal cortex which has been implicated in theory of mind, important for both social and communication behaviours (Abu-Akel and Shamay-Tsoory, 2011; Brink et al., 2011; Carrington and Bailey, 2009; Sabbagh, 2004; Völlm et al., 2006). BA41 corresponds to primary auditory cortex, of interest because of the increasing

recognition of the prevalence of sensory abnormalities in ASD (Kern et al., 2006; Klintwall et al., 2011; Leekam et al., 2007) as shown by their inclusion in the diagnostic criteria of DSM-5. Given that more sensory abnormalities are seen in those with high-functioning autism when compared to controls than in those with low-functioning autism when compared to controls (Leekam et al., 2007), our sample of only high functioning subjects provides a good opportunity to investigate potential differences in a primary sensory area. The most commonly reported sensory abnormality is auditory, specifically over-reactivity to sound (Klintwall et al., 2011), making primary auditory cortex a particularly good candidate to investigate.

Additionally, a VBM approach will be employed to provide a whole brain analysis of the data. Application of a VBM and an ROI-based approach will also allow the correspondence between findings from the two approaches to be assessed.

Methods

Subjects

Subjects were a subset of subjects included in previous studies on timing sensitivity in ASD and for whom timing measures had been previously acquired and were available for use in the present study (Falter et al., 2012a; Falter et al., 2012b). 11 male subjects with high-functioning autism or Asperger syndrome (17-42 years; 2 subjects with high functioning autism, 9 with Asperger syndrome) were compared with 11 typically developing controls (1 female; 16-38 years). Diagnosis of autism was confirmed with Autism Diagnostic Interview – Revised (ADI-R) and Autism Diagnostic Observation Schedule – Generic (ADOS-G). Verbal, performance and full scale IQ scores were

measured using the Wechsler Abbreviated Scale of Intelligence. ADI-R, ADOS-G and IQ measures had already been obtained as part of previous studies (Falter et al., 2012a; Falter et al., 2012b) (Table 2.2). Groups did not differ significantly on age or IQ (all $p>0.6$). Structural MRI scans had already been obtained but not analysed for eight ASD and ten control subjects as part of the previous study. Four additional structural scans (one control and three ASD subjects) were acquired as part of the present study (gap between scans was approximately 24 months).

| | ASD | | | Controls | | |
|-----------|------|-----|--------|----------|-----|---------|
| | Mean | SD | Range | Mean | SD | Range |
| Age (yrs) | 27.0 | 9.1 | 17-42 | 27.2 | 7.1 | 16-38 |
| VIQ | 115 | 11 | 89-127 | 117 | 15 | 99-139 |
| PIQ | 114 | 11 | 92-128 | 117 | 11 | 104-136 |
| FIQ | 116 | 10 | 89-131 | 119 | 12 | 101-141 |
| ADI-A | 15 | 5 | 9-26 | | | |
| ADI-B | 15 | 4 | 9-21 | | | |
| ADI-C | 6 | 3 | 2-12 | | | |
| ADOS-A | 3 | 1 | 1-5 | | | |
| ADOS-B | 6 | 2 | 1-9 | | | |
| ADOS-C | 1 | 1 | 0-3 | | | |

Table 2.2. Subject demographics. VIQ=verbal IQ; PIQ=performance IQ; FIQ=full scale IQ; ADI-A=ADI-R reciprocal social interaction domain; ADI-B=ADI-R communication domain; ADI-C=ADI-R restricted and repetitive behaviours domain; ADOS-A=ADOS-G communication domain; ADOS-B=ADOS-G reciprocal social interaction domain; ADOS-C=ADOS-G restricted and repetitive behaviours domain.

Scan acquisition

T1-weighted structural scans were acquired with a 1.5T clinical scanner (Siemens) with a head array coil with the following parameters: repetition time =12ms, echo time = 5.65 ms, flip angle=19 degrees, slice thickness = 1mm, in-plane resolution = 1mm².

Scan analysis

Scans were converted from DICOM format to NII files using dcm2nii (Rorden) prior to analysis using tools in the FMRIB software library (FSL) (Smith et al., 2004; Woolrich et al., 2009).

Automated identification of subcortical ROIs

FIRST was used to estimate volumes of left and right amygdala, caudate, putamen and pallidum. FIRST performs a two stage registration process where the whole head image is first registered to a non-linear MNI152 template using 12 degrees of freedom (dof). This is then used to initialise a second 12 dof registration to the MNI152 template using a subcortical mask. The inverse transformation is then applied to the modelled subcortical structures, allowing subsequent analysis to be performed in native space using original voxel intensities (Patenaude et al., 2011). Volumes of each of these subcortical structures could then be calculated (Figures 2.1, 2.2).

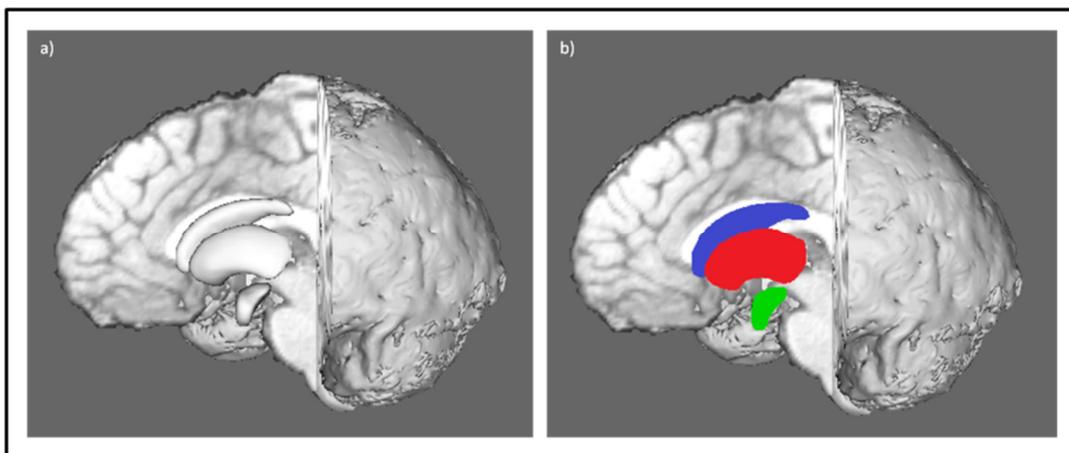


Figure 2.1. Example segmented subcortical structures (a); colour coded in b): red= putamen; blue= caudate; green= amygdala.

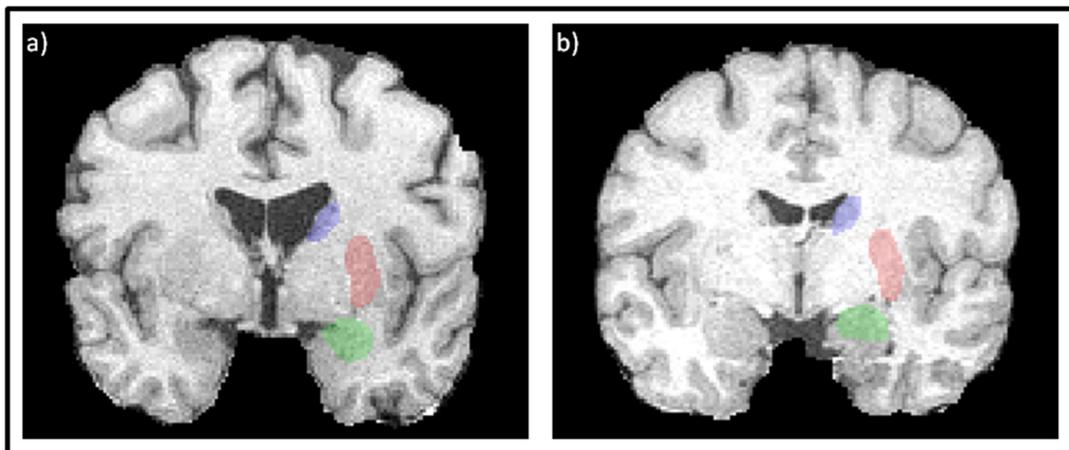


Figure 2.2. Segmented subcortical structures on example control (a) and ASD (b) brains, colour coded as for Figure 1 (red= putamen; blue= caudate; green= amygdala).

Localised shape differences between groups were investigated using vertex analysis as part of FSL's FIRST analysis. This creates an average shape across both groups, then finds the distance of each vertex location to this average shape for all subjects individually. These distances are then analysed by non-parametric permutation analysis with randomise (part of FSL) to test for group differences.

Manual identification of cortical ROIs

Images were run through BET (Smith, 2002) to remove all non-brain tissue. Estimates of grey matter (GM) and white matter (WM) volumes were calculated using FAST. FAST segments the brain into GM, WM and cerebral spinal fluid (CSF) based on a hidden Markov random field and expectation-maximisation algorithm (Zhang et al., 2001). Cortical regions of interest (ROIs) were traced on the structural scans according to the following anatomical definitions, and the volume of these areas measured.

Primary auditory cortex has been shown to follow the shape of Heschl's gyrus (Da Costa et al., 2011) which is bounded medially by the insula and laterally by the planum temporal. Heschl's gyrus can take several shapes, a single gyrus bordered by the first transverse sulcus and Heschl's sulcus, a single gyrus partially divided along its length by the sulcus intermedius or a completely divided gyrus where the sulcus extends down to the medial base of Heschl's gyrus leaving two parallel gyri (Da Costa et al., 2011). Although a recent in vivo study of primary auditory cortex on the basis of fMRI responses to pure tone stimuli has found that primary auditory cortex spans both gyri when multiple gyri are present (Da Costa et al., 2011), cytoarchitectural studies have found primary auditory cortex to be confined to the anterior-most gyri or the anterior part of the gyri if it is partially divided (Rademacher et al., 1993). This definition has been used in previous studies of BA41 volume (Hall et al., 2003; Penhune et al., 1996), and so

was used in the present study with the intermediate sulcus forming the posterior boundary of BA41 (Figures 3, 4).

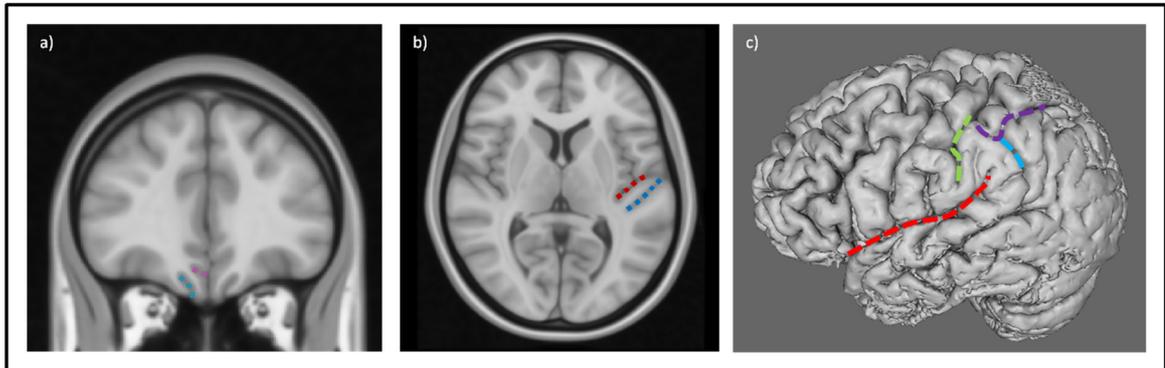


Figure 2.3. Location of sulci used in delineating cortical regions of interest. a) Sulci bordering BA11: purple= superior orbital sulcus, turquoise= olfactory sulcus; b) sulci bordering BA41: red=first transverse sulcus, blue=Heschl's sulcus; c) sulci bordering BA40: red=Sylvian fissure, green=post-central sulcus, purple=intraparietal sulcus, blue=Jensen sulcus.

BA11 was defined as the gyrus rectus, bounded medially by the midline, posteriorly by BA25, separated laterally from BA10 by the olfactory sulcus and dorsally from BA12 by the superior orbital sulcus. BA40 was defined as the supramarginal gyrus, bounded superiorly by the intraparietal sulcus, inferiorly by the sylvian fissure, anteriorly by the postcentral sulcus and posteriorly by the Jensen sulcus. The medial boundary was defined as the medial limit of the intraparietal sulcus (Figures 2.3, 2.4).

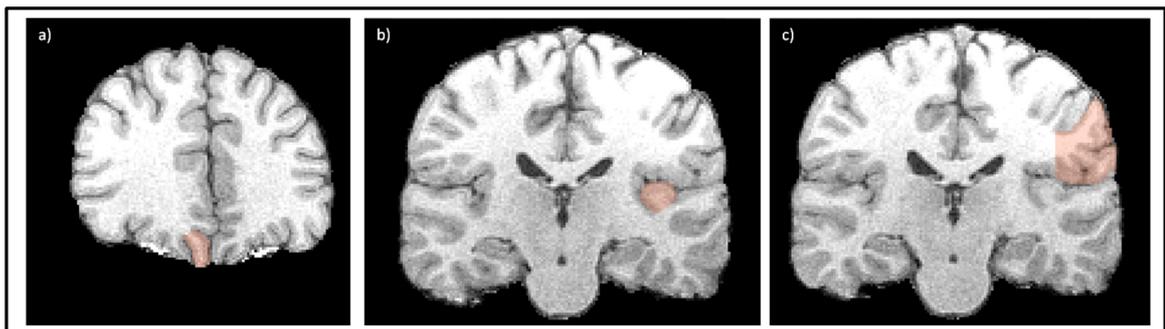


Figure 2.4. Example masks for a) BA11; b) BA41 and c) BA40

Measurements of the total ROI volumes were made, along with separate estimates of the grey and white matter contributions, based on segmentations produced by FAST.

VBM

Localised differences in grey matter volume were investigated using FSL-VBM (Douaud et al., (2007): <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM>). This is based on the VBM protocol developed by Good et al. (2001) and implemented in FSL (Smith et al., 2004). Brain extraction and segmentation of grey matter is performed on the structural scans. In order to be able to compare the scans they are transformed into standard space using non-linear registration (Andersson et al., 2007). A study-specific grey matter template was created by averaging these registered images. This averaged image was flipped along the x-axis and re-averaged to ensure left-right symmetry of the template image.

The original, non-registered grey matter images are then non-linearly registered to the grey matter template. The images are ‘modulated’; that is, each region is corrected for the relative contraction or expansion it has experienced due to the registration. For example, if a region is made twice as large, the intensity of that region is halved to maintain information about the total amount of grey matter when going from the original to modulated images.

The modulated images were smoothed with an isotropic Gaussian kernel with a sigma of 4mm. The result of this is that each voxel contains the average grey matter concentration of the surrounding voxels (with the number of voxels averaged over determined by the size of the kernel) (Ashburner and Friston, 2000). This means each voxel now contains a measure of local grey matter density, allowing voxel-wise comparisons to be carried out between groups. This voxel-wise comparison is implemented as a GLM using permutation-based non-parametric testing to account for the non-Gaussian distribution of the voxel values. Threshold-free cluster enhancement is used to control for multiple comparisons.

Timing measures

Measures of both event timing and interval timing were investigated as the present sample had previously been shown to differ from controls on measures relating to both forms of timing (Falter et al., 2012a; Falter et al., 2012b). Measurements of timing sensitivity had been collected as part of previous studies (Falter et al., 2012a; Falter et al., 2012b). Interval timing measures were available for eight subjects with ASD and seven controls; event timing measures were available for all eleven subjects with ASD and ten controls.

Interval timing

Both visual and auditory stimuli were used in this task. Visual stimuli were presented on a computer monitor and were white squares in the centre of a black screen. Auditory stimuli were 500Hz tones presented via headphones. Each trial consisted of presentation of a standard stimulus (for either 600 or 1000ms) followed by a random inter-stimulus interval of between 800 and 1300ms, and then a comparison stimulus. Participants were required to judge whether the comparison stimulus was the same or a different duration to the standard stimulus and respond by pressing one of two keys on a computer keyboard. For the 600ms standard stimulus, comparison stimulus durations were 300, 450, 600, 750 or 900ms and for the 1000ms standard stimulus, comparison stimulus durations were 500, 750, 1000, 1250 or 1500ms. In one third of the trials the comparison stimulus was the same duration as the standard stimulus and for the other two thirds it was different (higher for one third of total trials and lower for one third of total trials). The inter-trial interval was 300ms. Both standard and comparison stimuli could be either auditory or visual leading to four modality conditions (auditory-auditory (AA); visual-visual (VV); auditory-visual (AV) and visual-auditory (VA)). Blocks consisted of 12

trials and comparison durations were randomised within blocks. 16 blocks were presented and standard durations and modality were randomised between blocks. At the start of each block the modality which was going to be presented was indicated on the screen. No feedback was provided on performance throughout the task.

Signal detection analysis was applied to obtain measures of timing sensitivity (d'), the ability to distinguish between standard and comparison durations, and response bias (c), the tendency to reply 'same' across durations.

Event timing

Stimuli were presented on a computer screen with a black background. A white fixation cross was presented in the centre of the screen for the duration of each trial. 500ms after the beginning of the trial two vertical grey bars were presented on the screen either side of the fixation cross. The luminance of these bars was increased by steps (5.2, 12.8, 22.6, 34.2 and 53.7 lux), either simultaneously or with a stimulus onset asynchrony (SOA) of between 8.33 and 99.96ms (SOAs of 8.33, 16.66, 24.99, 33.32, 41.65, 49.98, 58.31, 66.64, 74.97, 83.30, 91.63 and 99.96ms). Participants were required to judge whether the bars appeared synchronously or asynchronously by pressing one of two keys on the computer keyboard. The session consisted of five blocks of 52 trials with each SOA presented 20 times with the order pseudo-randomised across the session. No feedback was provided on performance.

Response criterion and thresholds of simultaneity were calculated for each subject by fitting a least squares curve to the individual data sets. The steepness of the slope at the point of inflection gave the response criterion with a steeper slope indicating a sharper distinction between two categories. The point of inflection of the curve indicated the

sensory threshold of simultaneity as this is the point above which the subject is able to detect asynchronous stimuli presentations.

Statistical analysis

All statistical analysis was carried out in SPSS 19 for Windows. Group differences in ROI volumes were investigated using repeated measures ANOVAs (rmANOVAs) with region as within subjects variables and diagnosis as a between subjects variable. For investigation of cortical and subcortical ROIs, hemisphere was additionally included as a within subjects variable. Separate rmANOVAs were conducted for total grey and white matter, and cortical and subcortical ROIs to avoid violating assumptions of independence. For the same reason group differences in total brain volume were tested using an independent samples t-test. Interval timing measures were investigated using a rmANOVA, consistent with the analysis employed by Falter et al (2012b). Differences in event timing behaviour (response criterion and threshold of simultaneity) was compared using independent samples t-tests. Relationships with timing measures were investigated using Pearson's correlation analyses, given the small sample size included in these analyses, several correlations have been repeated using Spearman's Rank Correlation analyses to demonstrate that the same pattern of results was found, although the significance of the findings was reduced. Multiple comparisons were controlled for using Bonferroni corrections to adjust the statistical significance level by dividing by the number of statistical tests performed.

Results

Regional Differences

An independent samples t-test revealed no difference in total brain volume ($t=-1.293$, $p=0.211$). Diagnostic differences in grey and white matter volume were investigated using a rmANOVA with tissue type as the within subjects variable. This revealed a significant interaction between tissue type and diagnosis ($F(1,20)=9.154$, $p=0.007$), but no effect of tissue type ($F(1,20)=1.648$, $p=0.214$) or diagnosis ($F(1,20)=1.673$, $p=0.211$). Post-hoc t-tests revealed that the interaction was due to significantly larger grey matter volume in ASD compared to controls ($t=-2.280$, $p=0.034$) but no diagnostic difference in white matter volume ($t=0.144$, $p=0.887$)(Table 2.3). It was decided to investigate both absolute volume differences and volume differences corrected for brain size in order to investigate whether there are disproportionate differences in volume of any of the ROIs in ASD.

Diagnostic differences in the absolute volume of both subcortical and cortical ROIs was investigated using a rmANOVA with ROI and hemisphere as within subject factors, and diagnosis as the between subjects factor. This revealed a significant effect of region ($F(6,120)=326.239$, $p<0.001$) but no effect of hemisphere ($F(1,120)=0.472$, $p=0.500$) or diagnosis ($F(1,20)=0.049$, $p=0.826$) (Tables 2.3, 2.4). The same pattern of results was seen when correcting for total brain volume. However, given the significantly larger grey matter volume seen in ASD, grey matter volumes of cortical ROIs (corrected for total grey matter volume) were considered separately, using a rmANOVA with hemisphere and cortical ROI as within subjects factors and diagnosis as the between subjects factor. This revealed a significant effect of region ($F(1.265,20.984)=350.364$, $p<0.001$) and an interaction between ROI, hemisphere and diagnosis ($F(1.049,20.984)=4.359$, $p=0.048$),

| | Total volume (mm ³) | Grey matter (mm ³) | White matter (mm ³) | BA11 (mm ³) | | | BA40 (mm ³) | | | BA41 (mm ³) | | |
|-----|---------------------------------|--------------------------------|---------------------------------|-------------------------|----------------|----------------|-------------------------|-----------------|-----------------|-------------------------|---------------|---------------|
| | | | | L | R | Mean | L | R | Mean | L | R | Mean |
| TD | 1297969 (93591) | 639738 (64336) | 658231 (43429) | 3126 (710) | 3256 (977) | 3190 (795) | 15326 (3695) | 16138 (2518) | 15732 (2404) | 1912 (756) | 1752 (427) | 1832 (501) |
| ASD | 1355705 (114738) | 700727 (61061) | 654978 (60728) | 2575 (1092) | 2712 (1390) | 2643 (1188) | 16316 (5560) | 14488 (3745) | 15492 (4033) | 2095 (1108) | 1749 (487) | 1922 (738) |

Table 2.3. Mean (and standard deviation) of total brain volume, grey and white matter volume, and volume of the cortical ROIs for both ASD and control groups.

| | Amygdala (mm ³) | | | Caudate (mm ³) | | | Pallidum (mm ³) | | | Putamen (mm ³) | | |
|-----|-----------------------------|---------------|---------------|----------------------------|---------------|---------------|-----------------------------|---------------|---------------|----------------------------|---------------|---------------|
| | L | R | Mean | L | R | Mean | L | R | Mean | L | R | Mean |
| TD | 1567 (188) | 1456 (233) | 1512 (164) | 3871 (392) | 3762 (218) | 3817 (255) | 1833 (211) | 1884 (218) | 1858 (205) | 5581 (714) | 5493 (747) | 5537 (719) |
| ASD | 1604 (195) | 1535 (181) | 1570 (164) | 3944 (429) | 4051 (438) | 3998 (420) | 1952 (138) | 1960 (88) | 1956 (100) | 5584 (557) | 5520 (516) | 5552 (524) |

Table 2.4. Mean (and standard deviation) volume of subcortical ROIs for both ASD and control groups.

but no effect of hemisphere ($F(1,20.984)=0.020$, $p=0.889$) or diagnosis ($F(1,20)=1.407$, $p=0.249$). Post-hoc t-tests revealed smaller grey matter volume in left BA11 ($t=2.287$, uncorrected $p=0.033$) in ASD, however this did not survive correction for multiple comparisons (bonferroni corrected significance level for six post-hoc t-tests= 0.008).

No differences in localised structure shape were detected for any of the subcortical structures examined (all $p>0.5$).

VBM

VBM analysis revealed a large area of increased grey matter volume centred on the left occipito-parietal region although extending into the superior and inferior parietal lobes, in ASD subjects, which persisted when controlling for age (Figure 2.5).

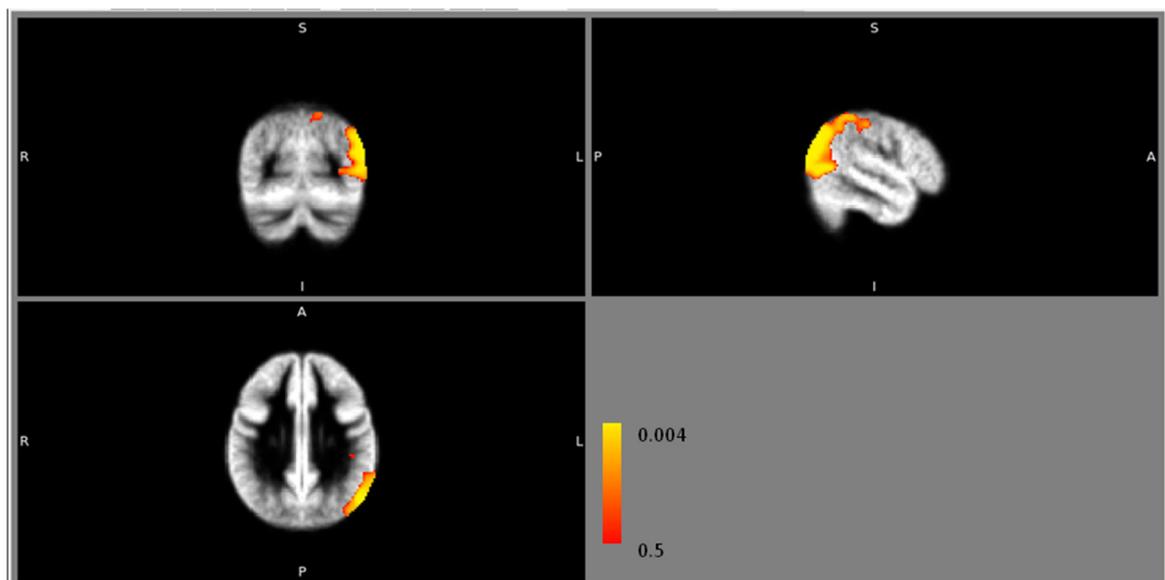


Figure 2.5. VBM results showing an area of increased grey matter volume in ASD compared to controls. Colour bar indicates corrected p-values.

Interval Timing

| | | Modality | | | | | | | |
|-----|----|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | | AA | | AV | | VA | | VV | |
| | | 600ms | 1000ms | 600ms | 1000ms | 600ms | 1000ms | 600ms | 1000ms |
| TD | d' | 2.99 (1.31) | 2.78 (1.72) | 1.48 (1.57) | 1.47 (1.71) | 1.69 (1.28) | 1.34 (1.21) | 1.83 (1.43) | 2.33 (1.91) |
| | c | -0.847 (0.837) | -0.657 (0.646) | -0.890 (0.747) | -0.751 (0.815) | -0.724 (0.622) | -0.283 (0.527) | -0.842 (0.788) | -0.736 (0.713) |
| ASD | d' | 1.75 (1.32) | 2.33 (1.44) | 1.21 (1.35) | 1.14 (1.25) | 0.98 (1.21) | 0.99 (1.42) | 1.89 (1.35) | 1.84 (1.59) |
| | c | -0.369 (0.820) | -0.490 (0.751) | -0.585 (0.745) | -0.397 (0.636) | -0.538 (0.493) | -0.266 (0.438) | -0.903 (0.869) | -0.769 (0.621) |

Table 2.5. Mean and standard deviations for timing sensitivity (d') and response bias (c) for ASD and control participants separately. Data had previously been collected and reported as part of a previous study and the data reported here represent a subset of that previously reported (Falter et al, 2012b).

Separate rmANOVAs with duration and modality as within subjects factors and diagnosis as the between subjects factor were conducted for timing sensitivity (d') and response bias (c) separately. A main effect of modality was seen for timing sensitivity only ($F(3,57)=7.673$, $p<0.001$), no other effects were seen for either timing sensitivity or response bias (all $F<2.7$, $p>0.12$) (Table 2.5).

Event Timing

| | Response Criterion | Threshold of Simultaneity |
|---------|--------------------|---------------------------|
| ASD | -2.56 (1.02) | 37.1 (13.8) |
| TD | -2.39 (1.25) | 41.3 (11.0) |
| t-value | 0.287 | 0.643 |
| p-value | 0.779 | 0.531 |

Table 2.6. Mean and standard deviations for response criterion and threshold of simultaneity for ASD and control participants separately. Data had been collected and reported as part of a previous study and the data reported here represent a subset of that previously reported (Falter et al, 2012a).

Independent samples t-tests were used to investigate diagnostic differences in response criterion and threshold of simultaneity separately. No significant differences were found (Table 2.6).

Correlations with timing measures

Given previous findings of involvement of basal ganglia and BA40 in interval timing, correlations with interval timing measures were restricted to investigation of the relationship between interval timing sensitivity and volume of these four areas. No correlations were found for controls although ASD subjects did show a correlation between interval timing sensitivity and left pallidum volume ($r= 0.623$, $p=0.040$, $n=11$, Bonferroni corrected significance level for 16 correlations= 0.003), but this disappeared if volumes were corrected for total brain size ($r=0.575$, $p=0.064$, $n=11$).

Event timing has been found to be associated with right inferior parietal lobe, therefore correlations of right BA40 volume with both response criterion and threshold of simultaneity were investigated. When controlling for brain size, controls showed a negative relationship between right BA40 volume and threshold of simultaneity (Pearson's $r=-0.775$, $p=0.041$; Spearman's $r=-0.643$, $p=0.119$; both $n=7$, Bonferroni corrected significance level for 4 tests= 0.013) that was still present if the analysis was restricted to grey matter (Pearson's $r=-0.793$, $p=0.034$; Spearman's $r=-0.714$, $p=0.071$; both $n=7$, Bonferroni corrected significance level for 4 tests= 0.013). However this relationship was absent in subjects with ASD (total right BA40 volume Pearson's $r=0.372$, $p=0.364$; Spearman's $r=0.381$, $p=0.352$; right BA40 grey matter volume Pearson's $r=0.283$, $p=0.498$; Spearman's $r=0.381$, $p=0.352$; all $n=8$, Bonferroni corrected significance level for 4 tests= 0.013) (Figure 2.6).

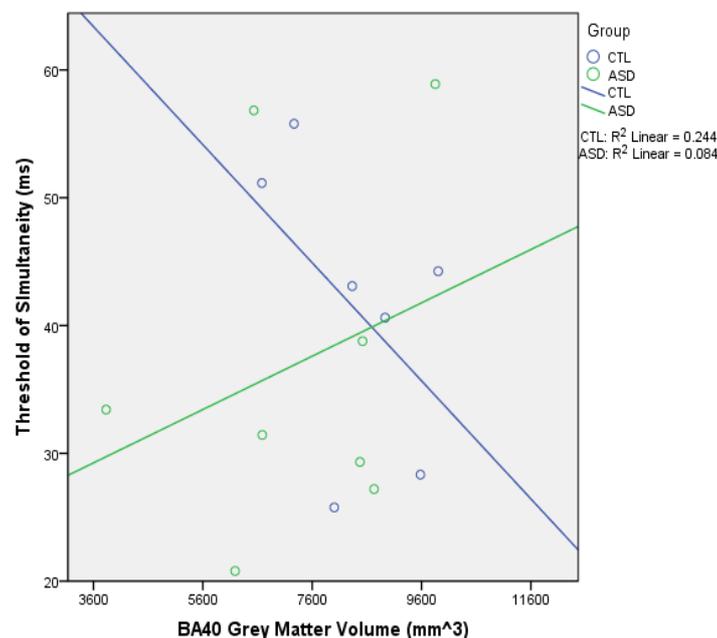


Figure 2.6. Relationship between grey matter volume in BA40 and thresholds of simultaneity. Controls $p=0.034$; ASD $p=0.498$

Investigation of relationships between timing measures and structural differences using VBM was restricted to measures of interval timing sensitivity, something previously

shown to be altered in ASD in a larger group of subjects including those included in the present study (Falter et al., 2012b). This was restricted to sensitivity in the auditory domain as ASD subjects were found to show reduced sensitivity particularly in this domain (Falter et al., 2012b), however no relationships were seen between auditory interval timing sensitivity and localised grey matter volume differences.

Correlations with demographic measures

As no correlations were seen between age and total brain volume (or grey matter and white matter separately) (all $p > 0.1$) absolute volumes of ROIs were used for correlation analysis. A positive correlation was seen between age and BA40 volume (Pearson's $r = 0.820$, $p = 0.002$; Spearman's $r = 0.747$, $p = 0.008$; both $n = 11$, Bonferroni corrected significance level for 6 tests = 0.008) in ASD subjects only, which survived correction for multiple comparisons, which was also seen when restricting the analysis to grey matter volume in BA40 (Pearson's $r = 0.810$, $p = 0.002$; Spearman's $r = 0.825$, $p = 0.002$; both $n = 11$, Bonferroni corrected significance level for 3 tests = 0.008) (Figure 2.7a). Further investigation of this relationship in the ASD subjects only found the correlation to be mainly driven by left BA40 grey matter volume (left Pearson's $r = 0.800$, $p = 0.003$; Spearman's $r = 0.706$, $p = 0.015$; right Pearson's $r = 0.496$, $p = 0.120$; Spearman's $r = 0.478$, $p = 0.137$; all $n = 11$).

ASD subjects also showed a positive correlation between age and BA41 volume ($r = 0.837$, $p = 0.001$, $n = 11$, Bonferroni corrected significance level for 6 tests = 0.008) which was also preserved when looking in grey matter only ($r = 0.740$, $p = 0.009$, $n = 11$, Bonferroni corrected significance level for 6 tests = 0.008), although this did not survive correction for multiple comparisons. In contrast control subjects showed a negative correlation between age and BA41 volume ($r = -0.750$, $p = 0.008$, $n = 11$, Bonferroni

corrected significance level for 6 tests=0.008) that was again apparent when restricting analysis to grey matter only ($r=-0.764$, $p=0.006$, $n=11$, Bonferroni corrected significance level for 6 tests=0.008) (Figure 2.7b). A similar pattern of results was seen if volumes were corrected for brain size.

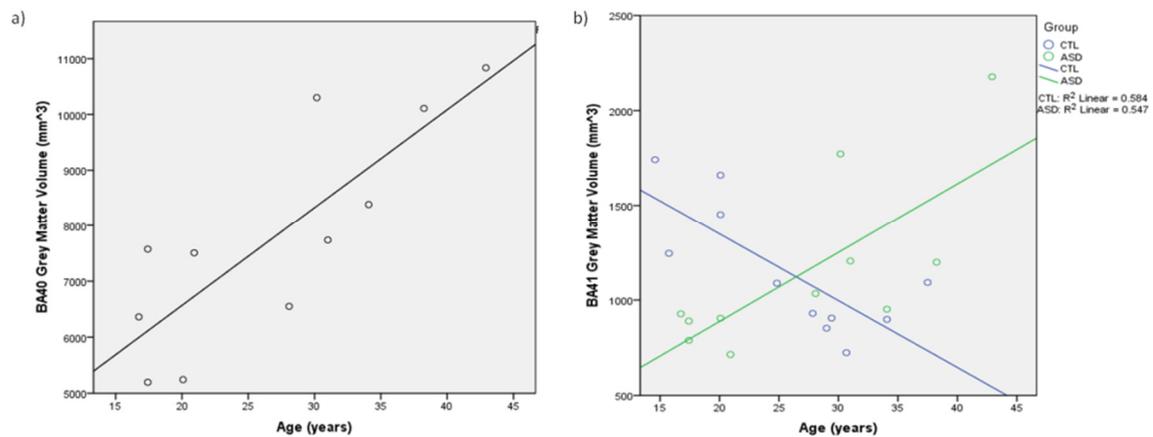


Figure 2.7. Least squares regression lines for the relationship between age and a) grey matter volume in BA40 in ASD only ($p=0.002$) and b) grey matter volume in BA41 in ASD ($p=0.009$) and controls ($p=0.006$).

BA41 volume was found to correlate negatively with ADI social and communication measures (ADI-A $r=-0.707$, $p=0.015$, ADI-B $r=-0.752$, $p=0.008$, both $n=11$). Visual inspection of the data (Figures 2.8a, 2.8b) suggested this might be due to two cases with large BA41 volumes. Exclusion of these cases reduced the significance of the relationship but did not abolish it (ADI-A $r=-0.685$, $p=0.042$, ADI-B $r=-0.749$, $p=0.020$, both $n=9$). BA11 volume was found to correlate negatively with social ability as measured by the ADOS (ADOS-B $r=-0.620$, $p=0.042$, $n=11$) (Figure 2.8c). However, none of these correlations survived correction for multiple comparisons (Bonferroni corrected significance level for 14 tests=0.004). A similar pattern of results was seen if volumes were corrected for brain size.

VBM analysis did not reveal any correlations with age, ADI subscale scores or verbal IQ.

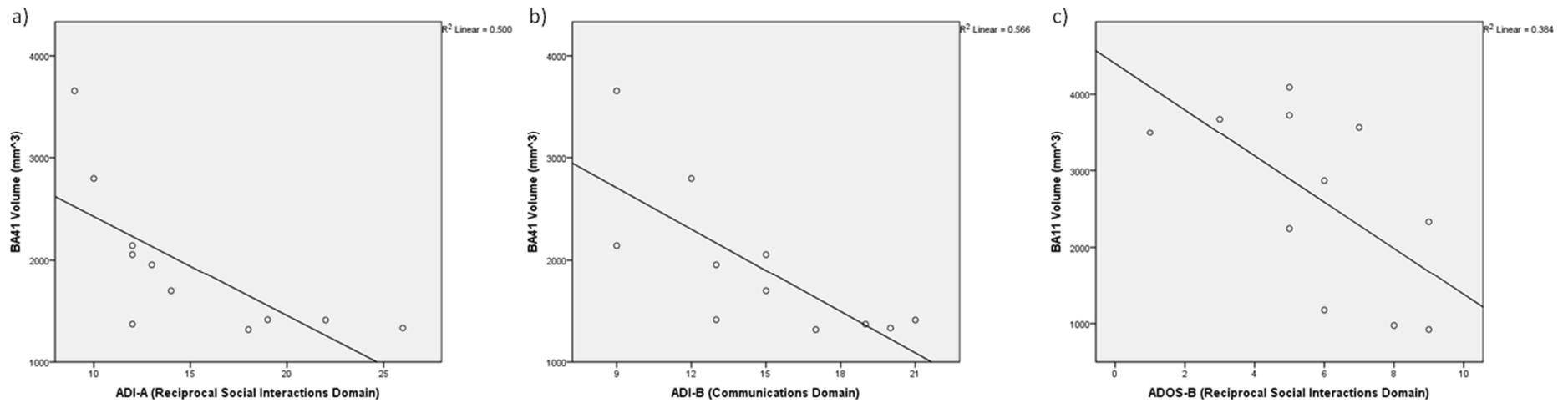


Figure 2.8. Least squares regression lines for the correlations between volumetric measures and social and communication scores. a) Correlation between BA41 volume and ADI-A scores ($p=0.015$); b) correlation between BA41 volume and ADI-B scores ($p=0.008$); c) correlation between BA11 volume and ADOS-B scores ($p=0.042$)

Discussion

Volumetric findings

The present finding of no difference in white matter and total brain volume between subjects with ASD and controls is consistent with previous findings in subjects in this age range (16-43 years) (Courchesne et al., 2011; Redcay and Courchesne, 2005). In contrast, the finding of increased grey matter volume in this age range is not so consistent with the previous literature. However, the present measure includes subcortical grey matter whereas most previous studies concentrated on cortical volume, finding decreases in ASD when compared to controls in adulthood (Wallace et al., 2010) (although one study reported increased cortical volumes in ASD in adolescence and early adulthood (Hazlett et al., 2006)). While the one study looking at cerebral grey matter volume found a negative relationship between cerebral grey matter volume and age, which would seem to be incompatible with the present findings, this study looked at children between 8 and 18 years (Lotspeich et al., 2004). It is possible that while cortical volume may be decreasing with age, and so would be expected to be smaller at the age range included in the present study, the subcortical structures may not be showing the same pattern. Indeed, previous studies have found increased caudate volume in ASD and absence of the age-related reduction of caudate volume seen in typically developing controls (Bonilha et al., 2008; McAlonan et al., 2002; Nickl-Jockschat et al., 2012; Rojas et al., 2006; Yu et al., 2011). Therefore it is possible that the increased grey matter volume in ASD subjects relative to controls is due more to differences in subcortical grey matter volume than cortical volume.

However, further analysis of the current data, excluding subcortical structures from the measures of grey matter volume, still found significantly larger cortical volumes in ASD

subjects ($t=-2.455$, $p=0.023$) with cortical volumes showing a strong positive correlation with total grey matter volumes for both ASD and control subjects (ASD $r=0.995$, $p<0.001$; control $r=0.997$, $p<0.001$). This suggests that subcortical structures contribute equally to total grey matter volume in both ASD and control subjects in this age range, which is consistent with our finding of no group differences in amygdala or basal ganglia volumes when controlling for brain size in the present study.

Despite finding no differences in the proportional volume of subcortical structures, the present study did find a trend towards larger absolute volumes of the right caudate in subjects with ASD. While not significant, this finding is consistent with previous findings of increased caudate volume in ASD in the absence of differences in other basal ganglia structures (Sears et al., 1999; Stanfield et al., 2008).

On investigating the grey matter volume changes in subjects with ASD further, we found reduced grey matter volume in left orbital frontal cortex. While this is not an area often reported to be changed at the volumetric level in ASD, not many studies employing an ROI-based approach have investigated orbital frontal cortex specifically. VBM studies have reported alterations in grey matter density in this region, with one study finding decreases in a group of 8-14 year olds (McAlonan et al., 2005), though other studies have found increases in both grey matter density (Bonilha et al., 2008) and cortical thickness (Hyde et al., 2010). However, the studies finding increases have generally older subjects (12-33 years, although the study by (Bonilha et al., 2008) also includes subjects from age 6 years). Studies performing ROI-based investigations of orbital frontal cortex volumes support this idea of age-dependent findings. In a study of children aged 8-12 years, Girgis et al. (2007) found a significant decrease of grey matter volume in ASD in right lateral orbital frontal cortex. Similarly, in subjects aged between 9 and 46 years, Hardan et al. (2006) found reduced right lateral orbital frontal cortex volume in children and

adolescents with ASD compared to controls, but increased volume of the right lateral orbital frontal cortex in adults with ASD compared to controls. However, neither study found significant differences in volumes of the medial orbital frontal cortex, which corresponds to the area investigated in the present study, although average volumes of medial orbital frontal cortex were lower in subjects with ASD than controls in the study by Girgis et al. (2007). However, there were also methodological differences in the definition of the medial orbital frontal cortex between the present study and those of Girgis et al. (2007) and Hardan et al. (2006). Whereas in the present study the superior limit was determined based on a line between the fundus of the superior orbital sulcus and the fundus of the olfactory sulcus, Girgis et al. (2007) and Hardan et al. (2006) defined the superior limit based on the level of the intercommisural line, which is likely to have resulted in larger estimates of the volume of the medial orbital frontal cortex. Although these methodological differences make it difficult to make direct comparisons with the ROI-based data, which finds no difference, VBM approaches finding differences in the medial orbital frontal cortex find decreases in children/adolescents and increases in adults, contradictory to what we have found in the present study.

VBM analysis revealed an area of increased grey matter volume in the ASD group, centred on the left occipital-parietal region but extending into the left inferior parietal lobe, which persisted when age was controlled for. Although most previous VBM analyses find differences in a greater number of areas our findings are consistent with previous findings looking at a similar demographic. The present sample is all high functioning (9 Asperger's, 2 high functioning autism) and previous studies investigating high functioning individuals find increased grey matter density in regions of inferior parietal lobe fairly consistently (Brieber et al., 2007; Ke et al., 2008; Yu et al., 2011). Further, the study by Yu et al. (2011) found that subjects with Asperger syndrome

showed increased grey matter in the left hemisphere, and that the increase was particularly pronounced in the inferior parietal lobe, consistent with what has been found in the present study.

While the region of increased grey matter volume identified in the VBM analysis encompassed BA40, the present study did not find a volumetric difference when employing a manual masking procedure. Despite not being statistically significant, the mean volume of left BA40 was higher in ASD when compared to controls, in contrast to right BA40 which actually showed a slightly lower mean. This pattern is also observed when looking at just the grey matter within the masked regions. Although both approaches show consistent differences, the ROI-based approach may not have been sensitive enough to detect the increases in volume. The area identified in the VBM analysis also identified regions superior and posterior to BA40 and did not cover the entirety of BA40, which could have reduced the size of the observed difference in the area identified by the manual mask, hiding an effect present in only part of the ROI.

Although differences were detected between ASD subjects and controls, these are perhaps fewer and more subtle than might have been expected from the existing literature. However, the ASD subjects in the current study mainly had Asperger syndrome, with the remaining two having high functioning autism. While these subjects were chosen in the hope of creating a more homogeneous group, structural differences have still been found between subjects with high functioning autism and Asperger syndrome, particularly in the thalamus and pallidum (McAlonan et al., 2008), meaning a lack of diagnostic difference in the present study could be due to conflicting findings in the two subgroups. However, there were too few subjects with high functioning autism to analyse alone, and exclusion of these two subjects from the ASD group did not change the findings in the ROI analysis.

Another reason why this study did not find many volumetric differences between ASD and control groups may be because the ASD group consisted of high functioning subjects. Previous studies have demonstrated fewer differences between controls and those with high functioning autism than are seen between controls and those with low functioning autism (Nordahl et al., 2007; Schumann et al., 2004).

Timing measures

Consistent with the findings of Falter et al (2012a), the present study found no diagnostic difference in response criterion in an event timing task. Although the present study was not able to replicate the finding of a significant difference in thresholds of simultaneity between ASD and TD groups found by Falter et al (2012a), the present study did show the same pattern, with higher thresholds being found in the TD group, suggesting that the smaller sample size (ASD=8; TD=7) may have prevented the present study from being able to detect diagnostic differences.

Similarly, although no diagnostic differences in interval timing behaviour were detected in the present study, the pattern of the data was consistent with that found by Falter et al (2012b), with TD participants showing higher scores for both timing behaviour (d') and response bias (c). Again, the smaller sample size may have prevented diagnostic differences from being detected in the present study.

Relationship with timing measures

No significant relationships were found between interval timing ability and volumes of either basal ganglia substructures or BA40. Prior investigation of processes involved in interval timing tasks revealed activity in different regions at different stages of the task (Harrington et al., 2004; Rao et al., 2001). The measure investigated in the current study,

ability to distinguish between standard and comparison intervals, is a product of both the ability to initially encode the standard interval and to correctly compare and judge whether the comparison interval is shorter or longer. Therefore, in order to find a relationship between timing sensitivity and volumetric differences it may be necessary to study the processes involved in completing an interval timing task individually. This may have also contributed to the lack of an association between interval timing sensitivity and volumetric differences in the VBM analysis.

The present study did find a significant negative relationship between volume of grey matter in right BA40 and a measure of event timing sensitivity, the threshold of simultaneity. This relationship was found in controls only, and was absent in subjects with ASD. A role for BA40 in event timing is consistent with previous studies which particularly implicated the right inferior parietal cortex in tasks using visual stimuli, such as in the present study (Lewandowska et al., 2010; Roberts et al., 2012; Woo et al., 2009). Although this relationship is not found in the ASD subjects in this sample, this may be due to the small sample size, the relative narrow spread of threshold of simultaneity values shown by the ASD subjects or may reflect the absence of this relationship in ASD. Consistent with the latter explanation is the finding of reduced thresholds of simultaneity in a previous study including these subjects (Falter et al., 2012b) and lower mean grey matter volume in right BA40 compared to controls in the present study.

Correlations with age

Although neither group showed significant changes in total brain or grey or white matter volume with age, there was a trend towards decreasing grey matter volume with age in the control group ($r=-0.509$, $p=0.109$) that was not present in the ASD group ($r=-0.068$,

$r=0.841$), consistent with previous findings of the absence of typical age related volumetric decreases in ASD (McAlonan et al., 2002; Raznahan et al., 2010).

Of the regions examined in the present study, the control group showed a significant decrease in grey matter volume with age only in BA41, consistent with previous findings of gradual grey matter volume decrease in primary auditory cortex in typical development (Sowell et al., 2003). In contrast, ASD subjects showed a positive correlation between grey matter volume in BA41 and age. While volumetric differences are not often reported in this region, ROI-based correlations with age are much less studied, especially in an adult sample. However, the presence of auditory processing abnormalities has been found to decrease with age in ASD but not in controls (Kern et al., 2006), suggesting altered functional ageing in ASD. To our knowledge this is the first study to investigate such a correlation in volumetric measures, providing evidence of altered ageing effects in BA41 between subjects with ASD and controls.

ASD subjects also showed a positive correlation between age and grey matter volume in BA40 which was more pronounced on the left hemisphere. This relationship was absent in controls. Such a discrepancy in ageing trajectories, particularly in left BA40 may contribute to the present finding of increased grey matter volume in that region in ASD subjects.

In contrast to the ROI-based analysis, the VBM analysis revealed no associations between age and localised increases in grey matter volume, either overall or in each group separately. This could reflect the fact that more comparisons are made in the VBM analysis, requiring stricter corrections to guard against false positives. Therefore any relationships present may not have survived the correction for multiple comparisons. However, relaxing the significance threshold to $p=0.1$ only revealed a age-related

reduction in grey matter volume in bilateral white matter in the internal capsule in the ASD group, suggesting this is not the only explanation for the discrepant findings.

Correlations with ASD severity

Consistent with previous findings, the present study found no relationship between clinical measures of symptom severity and total brain or grey or white matter volumes (Brieber et al., 2007). Negative correlations were observed between BA11 volume and social ability and BA41 volume and ADI measures of social and communication skills. Previous studies finding associations between cortical volumes and clinical measures are predominantly VBM studies, with the only study reporting an association finding greater BA41 grey matter density in a group with less severe symptoms compared to a group with more severe symptoms (Parks et al., 2009). A correlation between BA41 volume and communication scores in particular is of interest due to the importance of BA41 in language (Knaus et al., 2006) and suggests this may be an interesting area for future studies to follow up.

One previous study has investigated correlations between volume of the orbitofrontal cortex and ADI and ADOS social scores, however, associations were only found with white matter volumes in this region (Girgis et al., 2007). The study by Girgis et al. (2007) looked at children between 8 and 13 years of age, a developmental period when white matter volume has been found to be particularly large in ASD compared to controls (Herbert et al., 2003). This may explain the finding of a negative association with white matter in the study by (Girgis et al., 2007) and a negative correlation with grey matter in the present study when total white matter volume differences are not so pronounced. The finding of an association between social scores and BA11 volume is consistent with a study by (Powell et al., 2010) in typically developing adults which found that volume of

orbital frontal cortex correlated positively with social competence. Previous studies have also implicated this region in theory of mind which is important for social functioning (Abu-Akel and Shamay-Tsoory, 2011; Brink et al., 2011; Carrington and Bailey, 2009; Sabbagh, 2004; Völlm et al., 2006).

The absence of any correlations between symptom severity and subcortical structure volumes is perhaps surprising given previous findings associating amygdala volume with measures of social skills (Allely et al.; Barnea-Goraly et al., 2014; Munson et al., 2006; Schumann et al., 2009) and caudate volume with restricted and repetitive behaviours (Hollander et al., 2005; Sears et al., 1999). However, previous studies finding an association between amygdala volume and social functioning were all conducted in pre-adolescent children and most of them in children under 6. Given the findings of enlarged amygdala volumes in children but not necessarily adolescents (Schumann et al., 2004) it is possible that only amygdala volume in childhood meaningfully relates to social functioning.

Although one study found no association between measures of restricted and repetitive behaviours and caudate volumes (although they did find correlations with the volume of other basal ganglia structures) they only looked in children and so their results may not be comparable with the findings of the present study (Estes et al., 2011). Most studies implicating caudate volume in severity of restricted and repetitive behaviours have used a similar age range to the present study, although the small sample size in the present study compared to previous studies may have prevented any relationships from being detected.

ROI vs. VBM

Despite the advantages of VBM in providing an automated, reproducible, hypothesis-free approach to investigating local volumetric differences, there are also limitations to the approach. For example mis-registration between images due to differences in gyral or sulcal position or differences in cortical folding can affect the results of VBM (Ashburner and Friston, 2001; Giuliani et al., 2005; Kubicki et al., 2002). Studies directly comparing the results of ROI-based and VBM approaches to investigating structural differences between diagnostic groups suggest that VBM is not a replacement for the gold standard approach of manual ROI-based analysis, but that both techniques can reveal different, and sometimes complementary information (Giuliani et al., 2005; Kubicki et al., 2002).

Several studies have shown differences in the pattern of cortical sulci and cortical folding in ASD (Levitt et al., 2003; Nordahl et al., 2007) which could potentially affect the accuracy of registration and tissue classification during the VBM analysis (Ashburner and Friston, 2001). Such changes may also present an issue for cortical ROI-based analysis, especially given findings of alterations in position of the Sylvian fissure, superior temporal sulcus and interparietal sulcus which all form boundaries of BA40, and of the olfactory sulcus which forms one boundary of BA11 (Levitt et al., 2003). However, it is anticipated that this would cause less of a confound for the ROI-based approach utilised here. It is expected that shifts in sulcal position would either be meaningful with regards to cortical volume if they are either caused by or result from increases or decreases in the volume of neighbouring gyri, or irrelevant with regards to cortical volume if such an expansion or contraction of surface area was counterbalanced by a reduction in the cortical thickness. As sulci are used in delineating cortical ROIs

such shifts in sulcal position would therefore be expected to either result in no changes in cortical volume or reflect meaningful differences.

Limitations

The main limitation of the current study is the small sample size, especially given the expectation of more subtle differences when comparing those with high-functioning autism to controls (Nordahl et al., 2007; Schumann et al., 2004). In addition, the present sample covered a large age range. Although this is not expected to be as problematic as in childhood when volumetric differences are more pronounced (Courchesne et al., 2011; Redcay and Courchesne, 2005) different developmental trajectories in ASD subjects and controls may have affected the sensitivity to volumetric differences when comparing the two groups as independent samples, as was done in the present study.

Macroscopic approaches, as employed in the present study, are limited in their ability to provide details about the nature of the volumetric differences. For example, increased volume could be due to differences in the number or density of the neurons and which one of these is true could have implications for functioning of that area of the cortex. In addition, there could be changes at the cellular level which are not reflected in volumetric changes and so would be missed by macroscopic approaches. For example, there could be an increase in both numbers and density of neurons in a particular cortical area, giving rise to no volumetric difference.

Although the present study is limited by its inability to reproduce the differences in timing behaviour seen in the larger sample studied by Falter et al (2012a, 2012b), it is interesting that different relationships between timing behaviour and regional brain volumes are still seen in ASD and TD groups. This emphasises the fact that different

underlying processing may be going on in ASD in the absence of detectable differences in either structure or behaviour.

Conclusions

The present study found increases in grey matter in left parietal cortex and decreases in left BA11 in subjects with high-functioning ASD compared to controls. In contrast to controls who showed decreased grey matter in BA41 with age, subjects with ASD showed increased grey matter volume with age in both BA41 and BA40, consistent with the idea of altered developmental trajectories in ASD (Courchesne et al., 2011). Social and communication scores in subjects with ASD were found to correlate with volume of BA41 and BA11, an area implicated in theory of mind. The correlation between right BA40 volume and event timing sensitivity found in controls was not present in subjects with ASD, supporting a neural basis for altered timing sensitivity seen in ASD.

Chapter 3:

Minicolumn Study

Introduction

Although a more coherent story of an altered developmental trajectory is beginning to emerge from volumetric studies of ASD, investigations into the microstructural changes underlying such altered development have been less consistent.

Most histological studies of ASD have focused on investigations of neuronal size and number in the cortex. Reduced neuron size has been reported across a number of areas including the fusiform gyrus (van Kooten et al., 2008), anterior cingulate cortex (Simms et al., 2009), somatosensory cortex (Casanova et al., 2006a), primary motor cortex (Casanova et al., 2006a), primary visual cortex (Casanova et al., 2006a), BA44/45 (Jacot-Descombes et al., 2012), and prefrontal cortex (Casanova et al., 2006a) (although a study by (Courchesne et al., 2011b) found no difference in neuron size across prefrontal cortex). Several studies have found these differences in cell size to be age dependent with van Kooten et al. (2008) finding that the positive correlation seen between age and neuron size in the fusiform is absent in ASD. Similarly, Jacot-Descombes et al. (2012) report a negative correlation between neuron size in BA44/45 and age in controls but not subjects with ASD, meaning that the reduction in cell size seen in ASD in childhood is not present in adulthood. Reports of neuron density are more varied, however, with findings of reduced (van Kooten et al., 2008) or unaltered neuron density in the fusiform gyrus (Oblak et al., 2011), no difference in neuron density in the posterior cingulate cortex (Oblak et al., 2011), BA44/45 (Jacot-Descombes et al., 2012), or primary auditory cortex (Coleman et al., 1985), and unaltered (Mukaetova-Ladinska et al., 2004) or

increased neuronal density in the prefrontal cortex (Courchesne et al., 2011b). Given the findings of regional variation in volumetric differences, it is, perhaps, not surprising that similar regional variation has been found for measures of neuronal density. Although some of the discrepancy within both the prefrontal cortex and fusiform gyrus may be explained by considering developmental trajectory (i.e. both the studies finding no difference in neuron density were in adults (Mukaetova-Ladinska et al., 2004; Oblak et al., 2011), whereas studies finding alterations in neuron density incorporated both children and adults (Courchesne et al., 2011b; van Kooten et al., 2008)), it still does not help to resolve the difference in direction of the findings. Both the fusiform gyrus and prefrontal cortex are regions where the volumetric studies point reasonably consistently to decreased volume in children with ASD whereas the histological studies find one region showing increased neuronal density and the other decreased neuronal density. This suggests some inconsistency with differences in neuronal density not necessarily mirroring differences in overall volume. However, as can be seen from studies of volumetric changes, there is considerable heterogeneity between subjects, diagnostic groups (e.g. HFA vs. LFA), with age and with methodology, contributing to seemingly contradictory findings between studies. As of yet, relatively few studies have been conducted to investigate neuronal density in ASD, and those that have typically have fewer than ten ASD cases (Coleman et al., 1985; Courchesne et al., 2011b; Jacot-Descombes et al., 2012; Oblak et al., 2011; van Kooten et al., 2008), emphasising the need for more, larger studies before a coherent story can emerge.

One recent finding that seems to be consistent between all studies carried out so far, is the finding of reduced minicolumn width in ASD (Buxhoeveden et al., 2006; Casanova et al., 2006a; Casanova et al., 2002b; Casanova et al., 2002c; Casanova et al., 2006b). Minicolumns consist of a vertical string of 80-100 neurons stretching between layers II-

VI, with associated dendrites and myelinated axon bundles, and form the fundamental structural unit of the cortex (Buxhoeveden and Casanova, 2002; Casanova et al., 2008; Mountcastle, 1997). The core area of the minicolumn, containing the cell bodies, apical dendrites and unmyelinated axons, is surrounded by a cell sparse peripheral neuropil space (Casanova et al., 2002b). Multiple individual axons group together, forming bundles as they descend from layers III to VI within, or closely adjacent to, the core of the minicolumn (Casanova et al., 2002b). The edges of the minicolumn core contain GABAergic interneurons which are thought to provide inhibition between neighbouring minicolumns to ensure functional segregation (Favorov and Kelly, 1994). Minicolumn width, defined as the centre-to-centre spacing of the minicolumns, has been shown to be sensitive to regional variation within the brain (Chance et al., 2011) as well as both ageing (Chance et al., 2006; Chance et al., 2011; Di Rosa et al., 2009) and pathology (Casanova et al., 2002b; Chance et al., 2008; Di Rosa et al., 2009).

Findings of differences in minicolumn width have great explanatory potential in light of observed and suggested connectivity abnormalities in ASD. In light of findings of increased brain volume and decreased minicolumn width, it has been suggested that this would result in a huge increase in minicolumn numbers and therefore the fibres connecting these extra minicolumns. Due to the increased metabolic demand of longer axons, this increased white matter might therefore be biased towards local, short-range connections (Casanova et al., 2006b) explaining the increase in radiate white matter seen in children with ASD (Herbert et al., 2004). Measurements of intrinsic cortical connectivity using cortical separation distances to estimate the wiring costs between separate regions of the cortex, reveal reduced cortical separation distances in ASD in a range of cortical areas (pre- and post-central gyrus, primary motor cortex, primary somatosensory cortex, temporal lobe, parietal regions including the temporal parietal

junction, and orbital frontal cortex) suggestive of narrower minicolumns and more local connectivity.

Despite the large volumetric differences found in white matter, few studies have investigated the microstructural changes. Although a number of studies have suggested there may be altered myelin in ASD, these studies have either focused on levels of myelin associated proteins (Koul, 2006), antibodies against myelin basic protein (Mostafa and Al-Ayadhi, 2011; Singh et al., 1993) or imaging techniques sensitive to changes in myelin (Hendry et al., 2006). Several histological studies have suggested the presence of dystrophic axons in ASD (Azmitia et al., 2011; Weidenheim et al., 2001). A quantitative study of axonal density in white matter underlying prefrontal cortical regions found fewer extra large axons in deep white matter and a high density of small axons in superficial white matter underlying the anterior cingulate cortex in ASD compared to controls (Zikopoulos and Barbas, 2010). This was suggested to reflect fewer long range connections, which would be subserved by the extra large axons, and more local connections, due to the increased density of small axons. This pattern was only observed in the white matter underlying the anterior cingulate cortex and not below BA22, BA11 or lateral prefrontal cortex. White matter underlying BA11 did show an increased G-ratio in ASD, the ratio of axon diameter to myelin thickness, indicating thinner myelin regardless of axon diameter (Zikopoulos and Barbas, 2010).

Several studies have found that it is specifically the neuropil space component of the minicolumn that is reduced in ASD (Buxhoeveden et al., 2006; Casanova et al., 2002b). In these studies, neuropil space is calculated as the difference between the centre-to-centre spacing of the minicolumns and the width of the core region (the core region being defined as the part of the column containing 90% of the cells (Casanova et al., 2002b)). Therefore, it has been suggested that reduction of the neuropil space corresponds to loss

of inhibitory GABAergic cells at the edge of the core region (Casanova, 2006). This would affect the excitation-to-inhibition ratio and therefore cortical development, and increased excitation could explain the high rate of seizures seen in ASD (Casanova et al., 2002a). However, computational models of minicolumnar organisation suggest that decreased lateral inhibition would lead to the formation of wider minicolumns (Gustafsson, 1997; Gustafsson, 2004). Wider minicolumns would result in less overlapping dendritic trees and so more discrete functioning of individual minicolumns (Chance et al., 2012) which would be consistent with the preserved or even enhanced featural processing seen in ASD (Happé, 1999).

In a study of minicolumnar organisation in the middle temporal gyrus (BA21), dorsolateral prefrontal cortex (BA9) and area Tpt (part of BA22 at the posterior end of the superior temporal gyrus and including the posterior part of the planum temporale) Casanova et al. (2002b) found reduced minicolumn width and neuropil space in ASD compared to controls. It is not possible to see whether all regions showed a similar decrease or whether this was more pronounced in some regions than others, as results for each region individually were not reported in this study. These overall findings were replicated in the same subjects by Casanova et al. (2002c), using the grey level index to provide a measure of distance between peaks of intensity in black and white images, which would correspond to the core of the minicolumns. This would therefore provide a centre-to-centre measurement of minicolumn width. The findings from this study agreed with the earlier findings of reduced minicolumn width in ASD, but again provided no details on the degree of reduction in the individual areas (Casanova et al., 2002c). In addition, although the studies by Casanova and colleagues included age as a covariate in their analysis, no investigation was made of possible age specific differences between

subjects with ASD and controls, something of interest due to previous findings of age dependent volumetric differences (e.g. Courchesne et al. (2011a)).

In contrast a study by Buxhoeveden et al. (2006) specifically investigated the effect of both age and brain region on differences between subjects with ASD and controls. They investigated minicolumn width in three regions of prefrontal cortex (dorsal, orbital and mesial) and primary visual cortex (BA17), in two ASD cases (aged 3 and 41 years old) and five control cases (aged 2, 21, 34, 44 and 75 years). When comparing the adult cases, reduced minicolumn spacing and neuropil space were found in ASD in all three frontal regions, but no differences were found in V1. In contrast comparison of the two children revealed narrower columns in mesial frontal cortex only in ASD. Unlike the typically developing cases where significantly narrower minicolumns were found in all three frontal regions at age two, the three year old ASD case only showed narrower minicolumns in the mesial frontal cortex when compared to the 41 year old case. As a result of these region specific differences, it has been suggested that higher order association areas may be affected in autism without differences in primary sensory cortices (Buxhoeveden et al., 2006). However, as can be seen, this was a very small study, including only two subjects with ASD and control cases with a wider age range than the ASD cases, so these results can only be considered very preliminary.

Three more recent studies by Casanova and colleagues (Casanova et al., 2006a; Casanova et al., 2010; Casanova et al., 2006b) have further investigated the effect of region on differences in minicolumn width between groups. In a study looking at primary motor cortex (BA4), primary visual cortex (BA17), dorsolateral prefrontal cortex (BA9) and primary somatosensory cortex (area 3b), narrower minicolumns were once again found in ASD compared to controls (Casanova et al., 2006a). A significant effect of region was found, although no interaction between region and diagnosis, despite

including several primary sensory areas as well as a higher order association area. However, prefrontal association cortex (BA9) did show a larger difference between ASD and control means (2.9 μ m) than the primary sensory regions (0.7-1.2 μ m). Another study of the same cases but looking at more cortical regions (frontopolar cortex, orbital frontal cortex, dorsolateral prefrontal cortex, primary motor cortex, primary somatosensory cortex, frontoinsular cortex, Broca's area, anterior cingulate cortex and primary visual cortex) found no overall diagnostic difference but did find an interaction between diagnosis and cortical region (Casanova et al., 2006b). Post hoc investigation revealed increased neuropil space in frontopolar cortex and anterior cingulate, with non-significant decreases in neuropil space in dorsolateral prefrontal and primary visual cortices. The finding of significant increases in neuropil space demonstrates regional heterogeneity, but also that reductions in all minicolumnar components are not necessarily a universal feature of ASD. A final study by Casanova et al. (2010) looking at the same nine cortical areas in the same subjects (with inclusion of one extra older pair of cases) found narrower widths of the core region of the minicolumns in ASD as well as an interaction between diagnosis and cortical region. Although the mean value for each cortical region was lower in ASD, this was not assessed for statistical significance. Again, although the effect of age was controlled for, in these studies by use of matched pairs, age related differences were not investigated even though their subjects ranged from 4 to 25 years, an age range that might be expected to show a difference given the findings of Buxhoeveden et al. (2006). Investigation of the effect of age on differences between ASD and controls may also shed light on the findings of increased neuropil spacing in the study by Casanova et al. (2006b).

Therefore, the aim of the present study was to investigate potential minicolumnar differences in ASD, with a particular focus on regional and age-related patterns of

differences. Four different cortical regions have been chosen for investigation, representing both association cortices and a primary sensory region, allowing us to directly test Buxhoeveden et al. (2006) suggestion that association cortices are affected while primary sensory cortices are unchanged. BA41, primary auditory cortex, will be used as an example of primary sensory cortex, due to the high rate of auditory abnormalities in ASD (Klintwall et al., 2011). The planum temporale (PT) has been included as an example of unimodal association cortex, and also because of its role in language which is known to be affected in ASD. The final two regions comprise heteromodal association cortex; BA11 and BA40 both of which are important in theory of mind (Abu-Akel and Shamay-Tsoory, 2011; Brink et al., 2011; Carrington and Bailey, 2009; Sabbagh, 2004; Völlm et al., 2006). In addition, this range of cortical regions will allow us to investigate the suggestion that there is reduced cortical differentiation in ASD (Voineagu et al., 2011; Ziats and Rennert, 2013). The present study also includes participants between 4 and 88 years to enable whether any differences between ASD and control cases are observed at all ages, or whether they might be due to altered developmental trajectories in the two groups.

Methods

Subjects

Fixed tissue from 28 ASD brains and 25 typically developing controls, matched as far as possible for age (age range 4-88) was obtained for BA11, BA40, BA41 and PT. Tissue was obtained through the Autism Tissue Program (ATP) and the Thomas Willis Oxford Brain Collection (TWOBC). Where available information on sex, hemisphere, brain weight and clinical characteristics were also recorded (Tables 3.1, 3.2).

| Case | Source | Diagnosis | Sex | Hemi- sphere | Age (years) | Brain Weight (g) | Minicolumn Analysis | | | | Axon Bundle Analysis | | | |
|----------|-----------|-----------|-----|-----------------|----------------|---------------------|---------------------|------|------|----|----------------------|------|------|----|
| | | | | | | | BA11 | BA40 | BA41 | PT | BA11 | BA40 | BA41 | PT |
| BTB-5308 | NICHD BTB | ASD | M | * | 4.5 | * | + | + | + | | | | | |
| AN14266 | Harvard | ASD | M | * | 5 | 1420 | + | + | + | | + | + | + | + |
| AN03221 | Harvard | ASD | M | * | 7 | 1560 | + | + | + | | + | + | + | + |
| AN03407 | Harvard | ASD | M | R | 7 | 1575 | + | | | | | | | |
| AN13961 | Harvard | ASD | M | L | 7 | 1610 | + | + | + | | + | + | + | + |
| AN16641 | Harvard | ASD | M | R | 9 | 1320 | + | + | | | | + | + | + |
| AN01293 | Harvard | ASD | M | L | 9 | 1690 | + | + | + | + | + | + | + | + |
| AN15326 | Harvard | ASD | M | * | 10 | 1680 | + | + | | | | | | |
| AN00754 | Harvard | ASD | M | R | 13 | 1470 | + | + | | | | | | |
| UK92185 | TWOBC | ASD | M | * | 14 | * | + | + | + | + | | | | |
| AN11143 | Harvard | ASD | M | * | 14 | 1770 | + | + | + | + | + | + | + | + |
| AN02736 | Harvard | ASD | M | * | 15 | 1390 | + | | | | | | | |
| 92_1014 | TWOBC | ASD | M | L | 17 | 1451 | + | + | + | + | | + | + | + |
| UK68593 | TWOBC | ASD | M | L | 19 | 1243 | + | + | + | + | | | | |
| AN07817 | Harvard | ASD | M | R | 19 | 1090 | + | + | + | + | + | + | + | + |
| AN00764 | Harvard | ASD | M | R | 20 | 1144 | + | + | | | | | | |
| UK27798 | TWOBC | ASD | M | R | 22 | 1606 | + | + | + | + | | | | |
| AN11180 | Harvard | ASD | F | * | 25 | 1310 | + | + | + | + | + | + | + | + |
| AN12457 | Harvard | ASD | F | R | 29 | * | + | + | + | | + | + | + | + |
| 99_1071 | TWOBC | ASD | F | R | 32 | 1265 | + | + | + | + | + | + | + | + |
| 98_1174 | TWOBC | ASD | M | L | 35 | 1512 | + | + | + | + | + | + | + | + |
| AN07770 | Harvard | ASD | F | * | 40 | 890 | + | + | + | | + | + | + | + |
| UK45353 | TWOBC | ASD | F | R | 44 | 1592 | + | + | + | + | | | | |
| UK42577 | TWOBC | ASD | F | L | 44 | 1412 | + | + | + | | | | | |
| AN19534 | Harvard | ASD | M | * | 45 | 1360 | + | + | + | + | + | + | + | + |
| UK27696 | TWOBC | ASD | F | L | 48 | 1126 | + | + | + | + | + | + | + | + |
| AN16096 | Harvard | ASD | M | R | 50 | * | + | + | + | | + | + | + | |
| UK31539 | TWOBC | ASD | F | | 60 | * | + | + | + | + | | | | |
| UMB4670 | NICHD BTB | CTL | M | * | 4 | * | + | + | | | | | | |
| UMB4203 | NICHD BTB | CTL | M | * | 7 | * | + | + | + | | + | + | + | |
| UMB4337 | NICHD BTB | CTL | M | * | 8 | * | + | + | + | | + | + | + | + |
| M3538M | NICHD BTB | CTL | F | * | 9 | * | + | + | | | | | | |
| b1947 | TWOBC | CTL | F | L | 10 | 1458 | + | + | + | + | + | + | + | + |

| Case | Source | Diagnosis | Sex | Hemi- sphere | Age (years) | Brain Weight (g) | Minicolumn Analysis | | | | Axon Bundle Analysis | | | |
|---------|--------|-----------|-----|-----------------|----------------|---------------------|---------------------|------|------|----|----------------------|------|------|----|
| | | | | | | | BA11 | BA40 | BA41 | PT | BA11 | BA40 | BA41 | PT |
| 98_1105 | TWOBC | CTL | M | R | 13 | 1650 | + | + | + | + | + | + | + | + |
| b0958 | TWOBC | CTL | F | R | 13 | 1530 | + | + | + | + | + | + | + | + |
| 05_137 | TWOBC | CTL | M | R | 16 | 1445 | + | + | + | + | + | + | + | |
| 97_1312 | TWOBC | CTL | F | L | 17 | 1450 | + | + | + | + | + | + | + | + |
| 93_1351 | TWOBC | CTL | M | L | 18 | 1540 | | + | + | + | + | + | + | + |
| c3569 | TWOBC | CTL | M | R | 18 | 1520 | + | + | + | + | + | + | + | + |
| 91_1134 | TWOBC | CTL | F | L | 21 | 1340 | + | + | + | + | + | + | + | + |
| c1384 | TWOBC | CTL | M | R | 25 | 1592 | + | + | + | + | + | + | + | + |
| b8959 | TWOBC | CTL | M | R | 30 | 1400 | + | + | + | | + | + | + | |
| 95_1348 | TWOBC | CTL | F | R | 35 | 1330 | + | + | + | + | + | + | + | + |
| 93_1086 | TWOBC | CTL | F | L | 39 | 1330 | + | + | + | | | + | + | |
| b8927 | TWOBC | CTL | F | L | 45 | 1210 | + | + | + | | + | + | + | |
| c1754 | TWOBC | CTL | M | L | 45 | 1240 | + | + | + | + | + | + | + | + |
| 103_13 | TWOBC | CTL | F | * | 48 | 1143 | + | + | + | + | | | | |
| b1277 | TWOBC | CTL | F | R | 49 | 1360 | + | + | + | + | + | + | + | + |
| 21_13 | TWOBC | CTL | F | R | 68 | 1295 | + | + | + | + | | | | |
| 10_71 | TWOBC | CTL | M | R | 68 | 1500 | | + | + | + | | | | |
| 59_08 | TWOBC | CTL | M | L | 73 | * | + | + | + | + | | | | |
| 10_93 | TWOBC | CTL | F | L | 82 | * | + | + | + | + | | | | |
| 10_68 | TWOBC | CTL | F | R | 88 | 1103 | + | + | + | + | | | | |

Table 3.1. Subject demographics and areas investigated. Tissue was not available for all cases for all regions (+ indicates the tissue was available). * Data not available; TWOBC=Thomas Willis Oxford Brain Collection; NICHD BTB= NICHD Brain and Tissue Bank, University of Maryland; Harvard=Harvard Brain Tissue Resource Centre.

| Case | Sex | Age (years) | Seizures | ADI-A | ADI-B | ADI-C | ADI-D |
|----------|-----|-------------|----------|-------|-------|-------|-------|
| BTB-5308 | M | 4.5 | * | * | * | * | * |
| AN14266 | M | 5 | N | * | * | * | * |
| AN03221 | M | 7 | N | 27 | 16 | 8 | 5 |
| AN03407 | M | 7 | N | * | * | * | * |
| AN13961 | M | 7 | N | 29 | 14 | 3 | 5 |
| AN16641 | M | 9 | Y | 24 | 13 | 4 | 5 |
| AN01293 | M | 9 | N | 26 | 12 | 5 | 4 |
| AN15326 | M | 10 | Y | 26 | 14 | 10 | 4 |
| AN00754 | M | 13 | Y | 28 | 12 | 3 | * |
| UK92185 | M | 14 | Y | 23 | 19 | 6 | 5 |
| AN11143 | M | 14 | Y | 13 | 17 | 5 | 5 |
| AN02736 | M | 15 | Y | * | * | * | * |
| 92_1014 | M | 17 | N | * | * | * | * |
| UK68593 | M | 19 | N | 26 | 12 | 2 | 5 |
| AN07817 | M | 19 | * | * | * | * | * |
| AN00764 | M | 20 | N | 27 | 13 | 6 | 4 |
| UK27798 | M | 22 | N | 10 | 11 | 5 | 2 |
| AN11180 | F | 25 | Y | * | * | * | * |
| AN12457 | F | 29 | Y | 30 | 21 | 12 | 5 |
| 99_1071 | F | 32 | N | * | * | * | * |
| 98_1174 | M | 35 | Y | 25 | 14 | 7 | 5 |
| AN07770 | F | 40 | N | 12 | 14 | 8 | 5 |
| UK45353 | F | 44 | N | * | * | * | * |
| UK42577 | F | 44 | N | 26 | 10 | 3 | 3 |
| AN19534 | M | 45 | N | 27 | 14 | 4 | 5 |
| UK27696 | F | 48 | N | 29 | 23 | 6 | 5 |
| AN16096 | M | 50 | * | * | * | * | * |
| UK31539 | F | 60 | N | * | * | * | * |

Table 3.2. Clinical characteristics of ASD cases. * indicates this information was not available.

Neurohistological sampling

Brains were sectioned coronally and blocks of size 25mm x 25mm x 10mm were sampled for each of the three regions from one hemisphere per brain. Blocks and the surrounding tissue were photographed using an Olympus C-5050 digital camera for reference. BA41 blocks incorporated Heschl's gyrus and the planum temporale. BA41 is bordered medially by the insula cortex and laterally by the planum temporale. The anterior boundary is marked by the first transverse sulcus and the anterior boundary by Heschl's sulcus. The planum temporale is located on the lower bank of the Sylvian fissure and bounded posteriorly by its ascending ramus. The anterior boundary is formed by Heschl's gyrus (Figures 3.1, 3.2; also see Figure 2.3). BA11 blocks were sampled from the gyrus rectus, bounded medially by the midline, posteriorly by BA25, separated

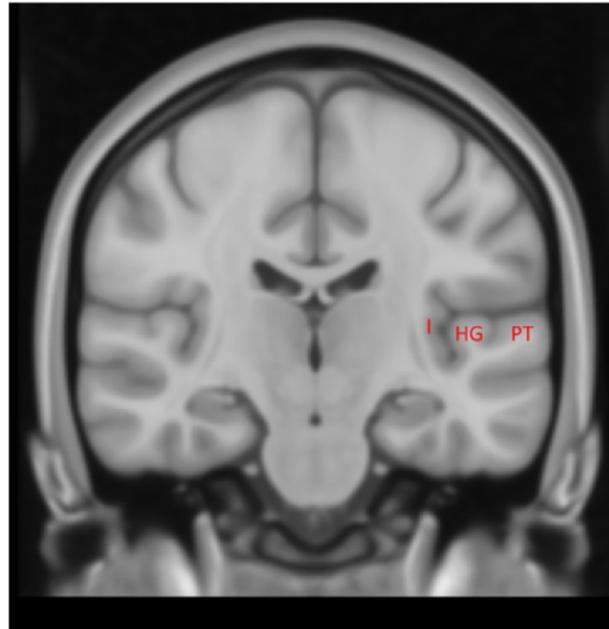


Figure 3.1. Anatomical boundaries used in the sampling of BA41 and PT. I=insula; HG=Heschl's gyrus; PT=planum temporale.

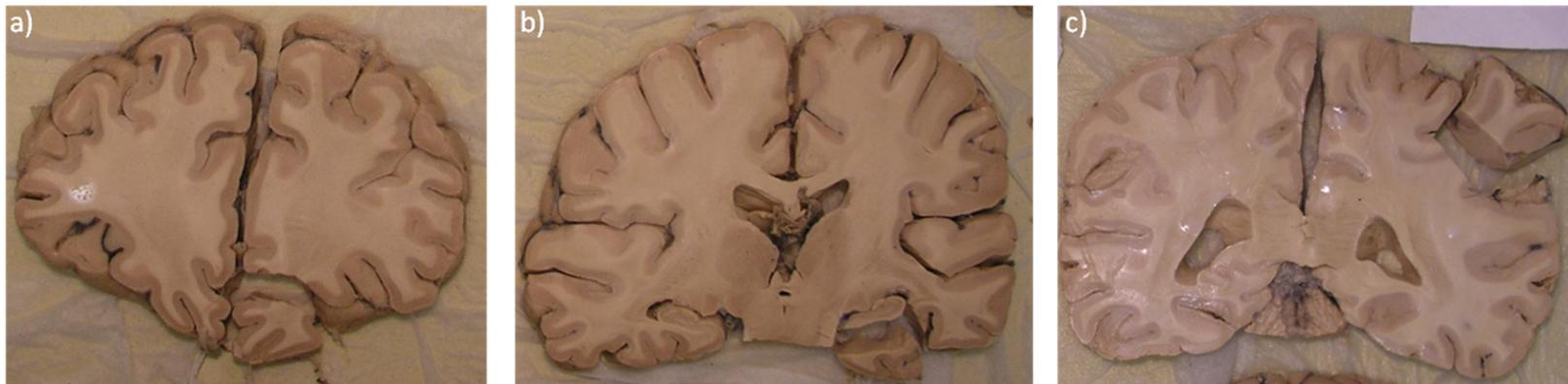


Figure 3.2. Example sectioning of blocks. a) BA11, b) BA41 and PT and c) BA40.

laterally from BA10 by the olfactory sulcus and dorsally from BA12 by the superior orbital sulcus (see Figures 3.2, 2.3). BA40 was defined as the supramarginal gyrus, which is bounded superiorly by the intraparietal sulcus, inferiorly by the sylvian fissure, anteriorly by the postcentral sulcus and posteriorly by the Jensen sulcus (see Figures 3.2, 2.3). We were particularly interested in area Pfm of BA40, which is defined cytoarchitecturally but is located towards the posterior part of the supramarginal gyrus. Therefore, where possible, BA40 was sampled from the area posterior to the end of the Sylvian Fissure. However, for cases obtained from the ATP, despite availability of this region being indicated by the online database (atpportal.org), for some cases this area was no longer available by the time the brains were sampled, resulting in the present study being provided with a mixture of the target region (area Pfm) and a more anterior part of BA40.

All tissue was sucrose protected (30% w/v) prior to freezing to minimise artefacts. Four 30-um sections were cut from each block. Two were Nissl stained with cresyl violet (CV; ThermoFisher Scientific, Waltham, MA, USA) for visualisation of neurons (Figure 3.3) and two were stained with Sudan Black for visualisation of axon bundles (Figure 3.4).

Minicolumn Analysis

Minicolumn width based on cell bodies was assessed using a semi-automated procedure that has been described in detail previously (Buxhoeveden et al., 2001; Casanova and Switala, 2005). This procedure gives a value for the minicolumn width consisting of the cell dense core region plus the associated neuropil space surrounding this. For each ROI, four pictures were taken, two from each slide where possible, each containing a region of about 1 mm². Image locations were selected using a random number generator, excluding areas of high curvature which have been shown to affect cell distribution (Chance et al.,

2004). As minicolumns are clearest in layer III, photographs were centred on that layer and obtained through a 4x objective lens, with an Olympus BX40 microscope. A semi-automated computerised method is used to measure aspects of minicolumn structure. Layer III is first manually defined on the photograph and analysis confined to this layer. The software then distinguishes between the stained cells and unstained background, applying a threshold to remove small fragments of 'noise'. This allows identification of the neurons comprising the core of the minicolumns and therefore the centre-to-centre spacing of the minicolumns can be calculated (more details can be found in Casanova and Switala (2005); Di Rosa et al. (2009) and Chance et al. (2004)). Values calculated from the four photographs were averaged to give a single value for each region.

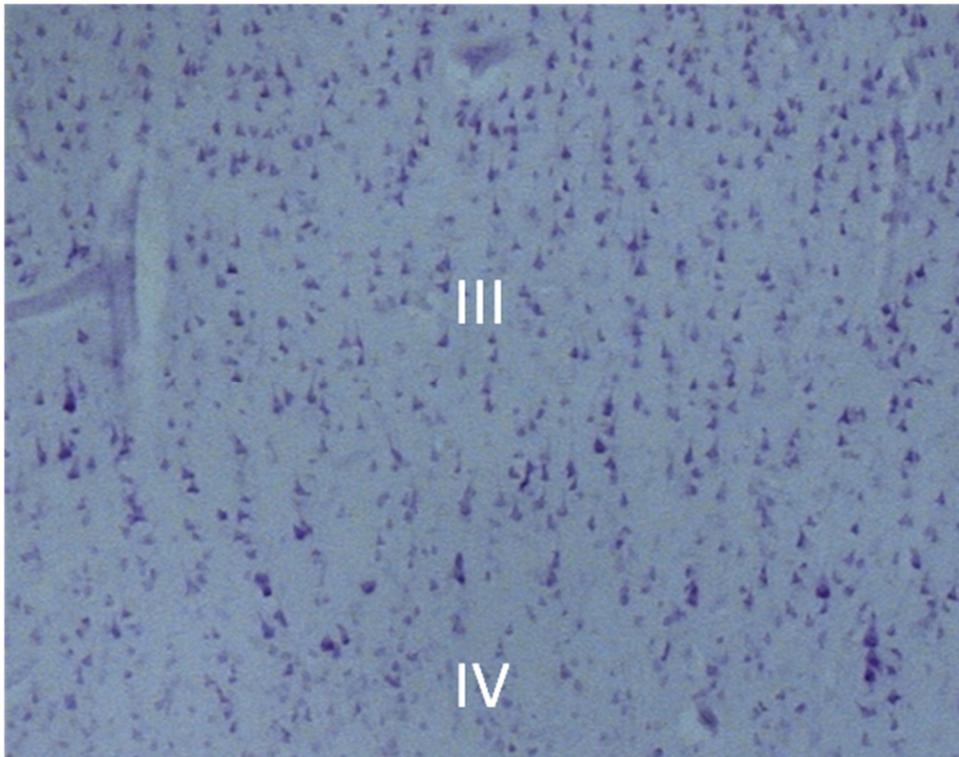


Figure 3.3. Example image from ASD primary auditory cortex used for minicolumn analysis with cortical layers labelled.

Axon Bundle Measurements

The method for measuring axon bundles was developed and optimised as part of the project described in Chapters 4 and 5. In order to obtain axon bundle measurements for this data set, I trained a student in the staining and image analysis, and supervised the collection of data. This data was then analysed alongside the minicolumn data as part of the following write-up.

For each region three photographs were obtained through a 10x objective lens (resolution $1.10\mu\text{m}$, although the resolution achieved with real data will be lower due to the presence of noise) with an Olympus BX40 microscope, centred around layer V as the axon bundles were clearest there. Areas of extreme curvature were avoided where possible, as was done for the minicolumn measurements. Measurements of axonal bundle centre-to-centre spacing, and the width of the bundles themselves were made manually in Axiovision, using the in-built measurement tools (Figures 3.4, 3.5). The digital resolution of the analysed images was $0.67\mu\text{m}/\text{pixel}$.

A sample line of standard length ($590\mu\text{m}$; determined by the size of the image view) was drawn across the centre of the photograph, perpendicular to the bundle direction in order to identify the bundles to be measured. Only bundles intersecting this line were measured, those that passed out of the plane of sectioning above or below the line were not included. Single axons or pairs of axons crossing the line were not considered to constitute axon bundles for the purposes of this analysis. Bundles (>2 axons) were identified and their centres marked (Figure 3.5).

Bundle spacing measurements were then made from the centre of each bundle marked in this way to the centre of the adjacent bundle, for all bundles intersecting this line. The width of each axon bundle was also measured. For the width measurements, the edges of

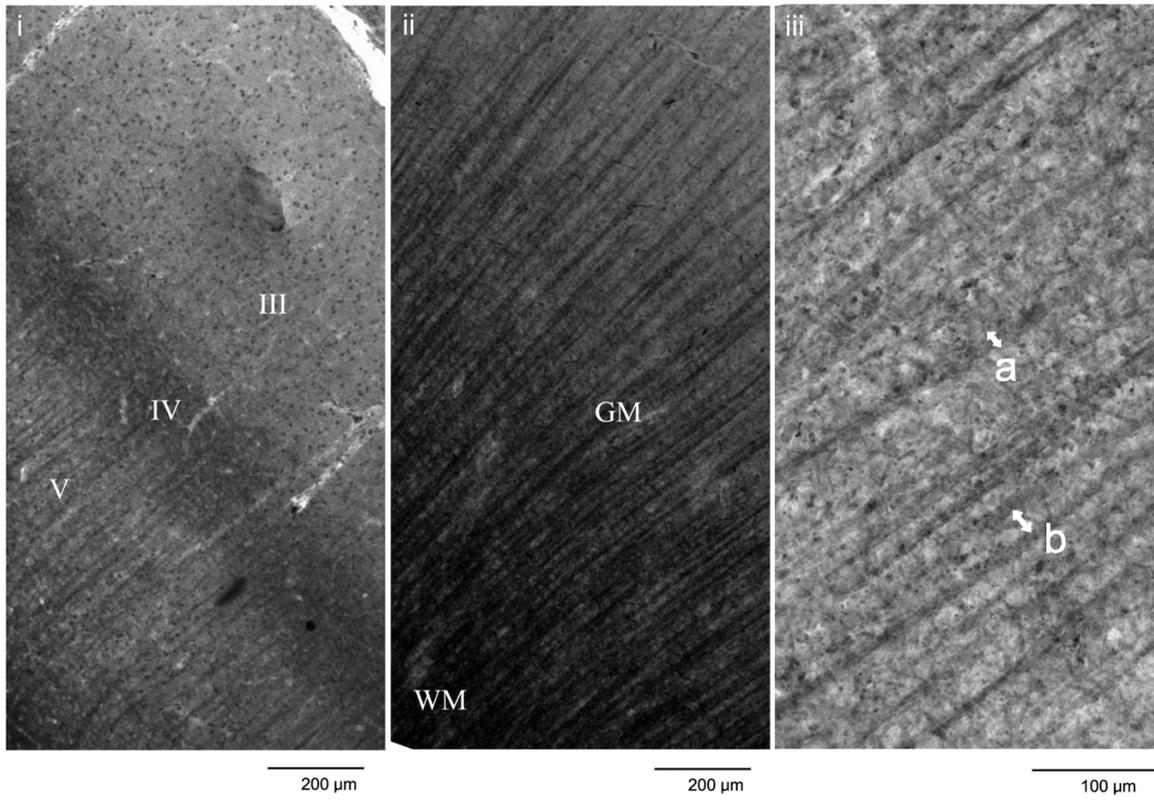


Figure 3.4. Sudan black stained section illustrating i) cortical layers, ii) tissue type and iii) measurements of axonal bundle width (a) and axonal bundle spacing (b), as indicated by the white arrows.

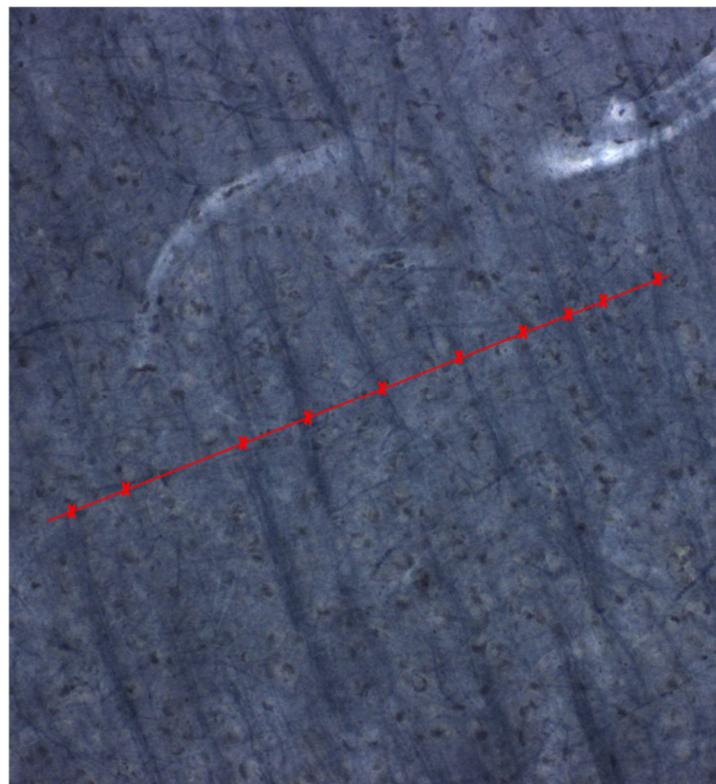


Figure 3.5. Example of axon bundles marked on the reference line

the bundles were marked at the point where they intersected the line, and the bundle width was determined as the distance between these two points. Edges of axon bundles were distinguished by the change in intensity of staining from the background, which identified the start of the more darkly stained axon bundle. Pilot data revealed high reliability of this method when comparing measurements of the same photographs made on different occasions (for bundle width ICC=0.81, $p<0.001$; for bundle spacing ICC=0.98, $p<0.001$). The values from the three photographs were then averaged to give a single value for bundle spacing and a single value for bundle width for each ROI.

Statistical analysis

All data were analysed using SPSS v19 for Windows.

Repeated measures ANOVAs with cortical region as the within subjects factor and diagnosis as the between subjects factor were used to investigate differences in minicolumnar width, axon bundle width and axon bundle spacing. The effect of including age and brain weight as covariates was also investigated.

Group differences in age and brain weight were investigated using independent samples t-tests. Pearson's correlation coefficient was used to investigate correlations.

Throughout the results section, statistical significance will be indicated by * for uncorrected $p<0.05$; and ** for uncorrected $p<0.01$.

Results

Minicolumn Data

Regional and Diagnostic Differences

A repeated measures ANOVA with cortical region as the within subjects factor and diagnosis as the between subjects factor revealed a significant effect of region ($F(3,84)=17.417$, $p<0.001$), as well as a significant effect of diagnosis ($F(1,28)=8.098$, $p=0.008$), with wider minicolumns being found in ASD (Table 3.3).

As can be seen from Table 3.1, minicolumn measurements for the PT were not available for all cases, so this analysis was repeated with the PT excluded. This revealed a significant effect of region ($F(2,82)=33.912$, $p<0.001$) and a trend towards an effect of diagnosis, although this did not reach significance ($F(1,41)=3.325$, $p=0.076$).

Post-hoc investigation of the effect of diagnosis revealed wider minicolumns in ASD in all regions, with significant differences observed in BA11 ($t(28)=2.942$, $p=0.006$) and PT ($t(28)=3.144$, $p=0.004$) and a trend towards a difference in BA41 ($t(28)=1.846$, $p=0.076$) (Bonferroni corrected significance level for 6 tests= 0.008) (Table 3.3). Post-hoc investigation of the effect of region found significant differences between all pairs of regions (all $p<0.008$) except BA11 and PT (Figure 3.6). Including brain weight and age as correlates in the model reduced the effect of cortical region (possibly due to a significant interaction between cortical region and brain weight, $F(2,007,42.151)=3.470$, $p=0.040$) though there was still a significant effect of diagnosis ($F(1,21)=6.957$, $p=0.015$).

| Diagnosis | Minicolumn Width (μm) | | | |
|-----------|------------------------------------|------------|------------|------------|
| | BA11 | BA40 | BA41 | PT |
| ASD | 34.8 (3.7) | 36.2 (5.1) | 32.1 (3.2) | 35.6 (2.9) |
| Controls | 32.5 (2.7) | 35.1 (3.6) | 30.4 (3.1) | 32.6 (3.1) |

Table 3.3. Mean (and standard deviation) for minicolumn widths in each cortical region for ASD and control cases.

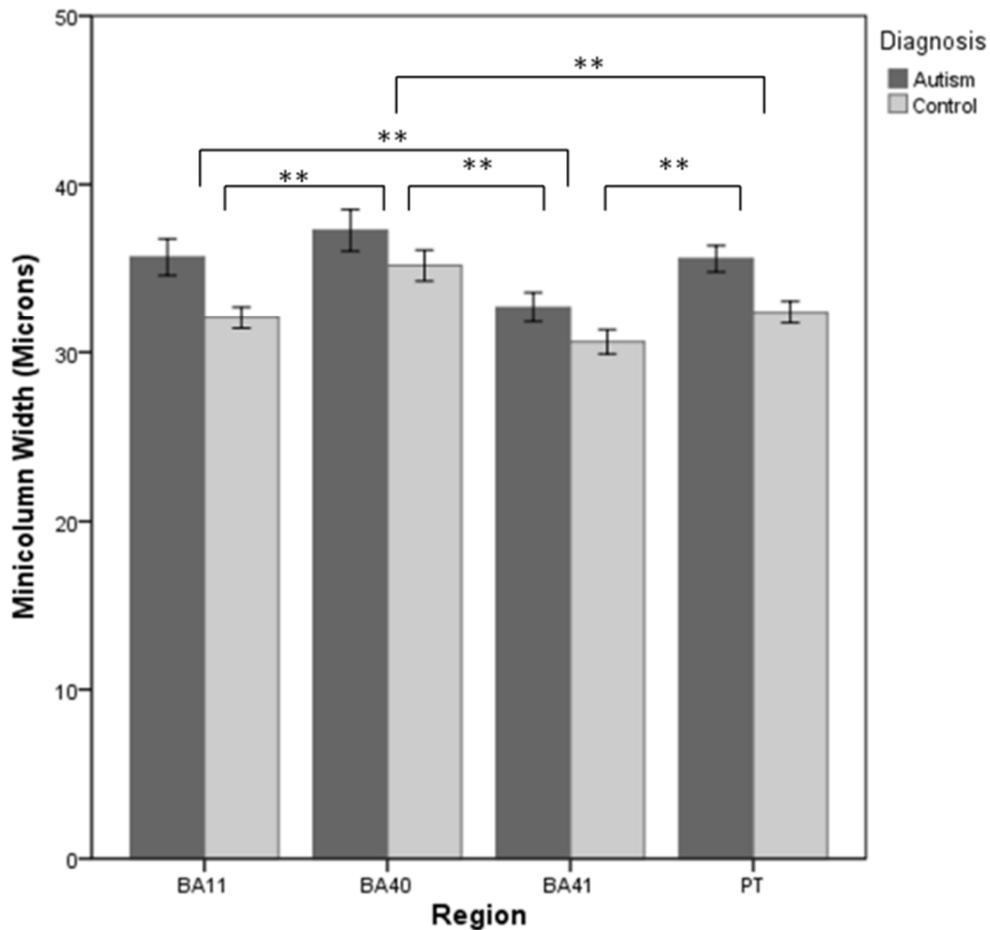


Figure 3.6 Average minicolumn width for ASD and control cases for each cortical region. Error bars indicate the standard error of the mean.

Although the finding of no interaction between diagnosis and cortical region suggests a similar pattern of regional differences in both ASD and controls, given our a priori interest in whether regional differentiation is attenuated in ASD, we addressed this question explicitly by conducting this analysis on each group separately. A repeated measures ANOVA, with cortical region as the within subjects factor, revealed a significant main effect of cortical region for both ASD ($F(3,39)=5.486$, $p=0.003$) and control ($F(3,45)=18.615$, $p<0.001$) groups separately. Post- hoc analysis of the effect of

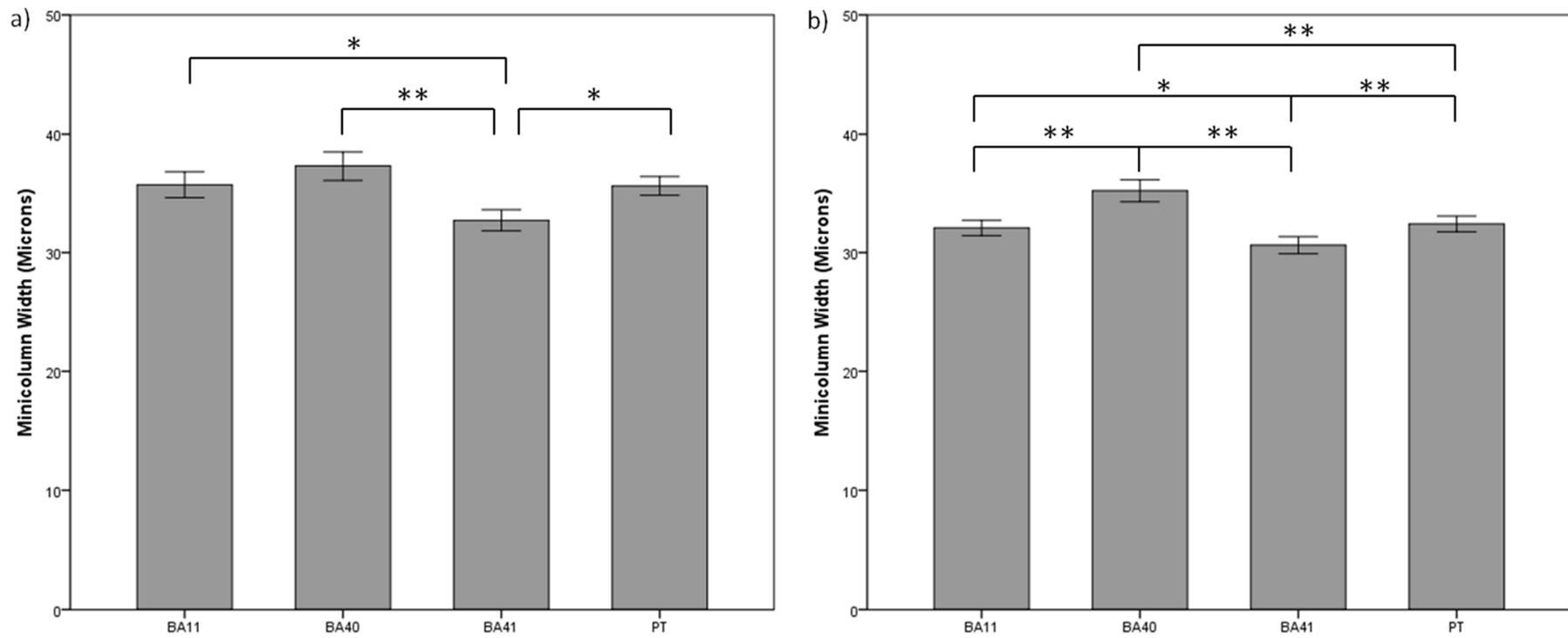


Figure 3.7. Regional differences in minicolumn width for a) ASD and b) control cases separately.

region revealed that while significant differences were seen in controls between all regions (all $p < 0.03$), except PT and BA11 ($p = 0.573$) (Bonferroni corrected significance level for 6 tests = 0.008), the ASD group showed differences only between BA41 and all other cortical regions (all $p < 0.02$) (Bonferroni corrected significance level for 6 tests = 0.008) (Figure 3.7).

Relationship with demographic measures

No differences were seen between diagnostic groups for either age or overall brain weight ($p > 0.05$), although brain weight was not available for all cases. In order to investigate relationships with demographic measures, an average minicolumn width was calculated for each case by averaging the different regional values. Although this is not ideal as regional differences were seen in minicolumn width, an average value was used in order to minimise the number of comparisons conducted. In addition, as was shown above, regional differentiation follows a similar pattern in both ASD and control groups. Two averages were calculated; one over BA11, BA40 and BA41, and one over BA11, BA40, BA41 and PT for those cases for which a value for PT was available.

A trend towards a negative correlation was observed between minicolumn width and age for ASD (excluding PT $r = -0.417$, $p = 0.054$, Figure 3.8a; including PT $r = -0.509$, $p = 0.063$, Figure 3.8c, 3.9), but this was less evident for control cases when PT was included ($r = -0.456$, $p = 0.088$, Figure 3.8d, 3.9) and absent when PT was excluded ($r = -0.254$, $p = 0.281$, Figure 3.8b). When regions were considered individually no significant correlations were observed between minicolumn width and age (all $p > 0.09$) although controls showed a trend towards a negative relationship in BA40 ($r = -0.387$, $p = 0.056$).

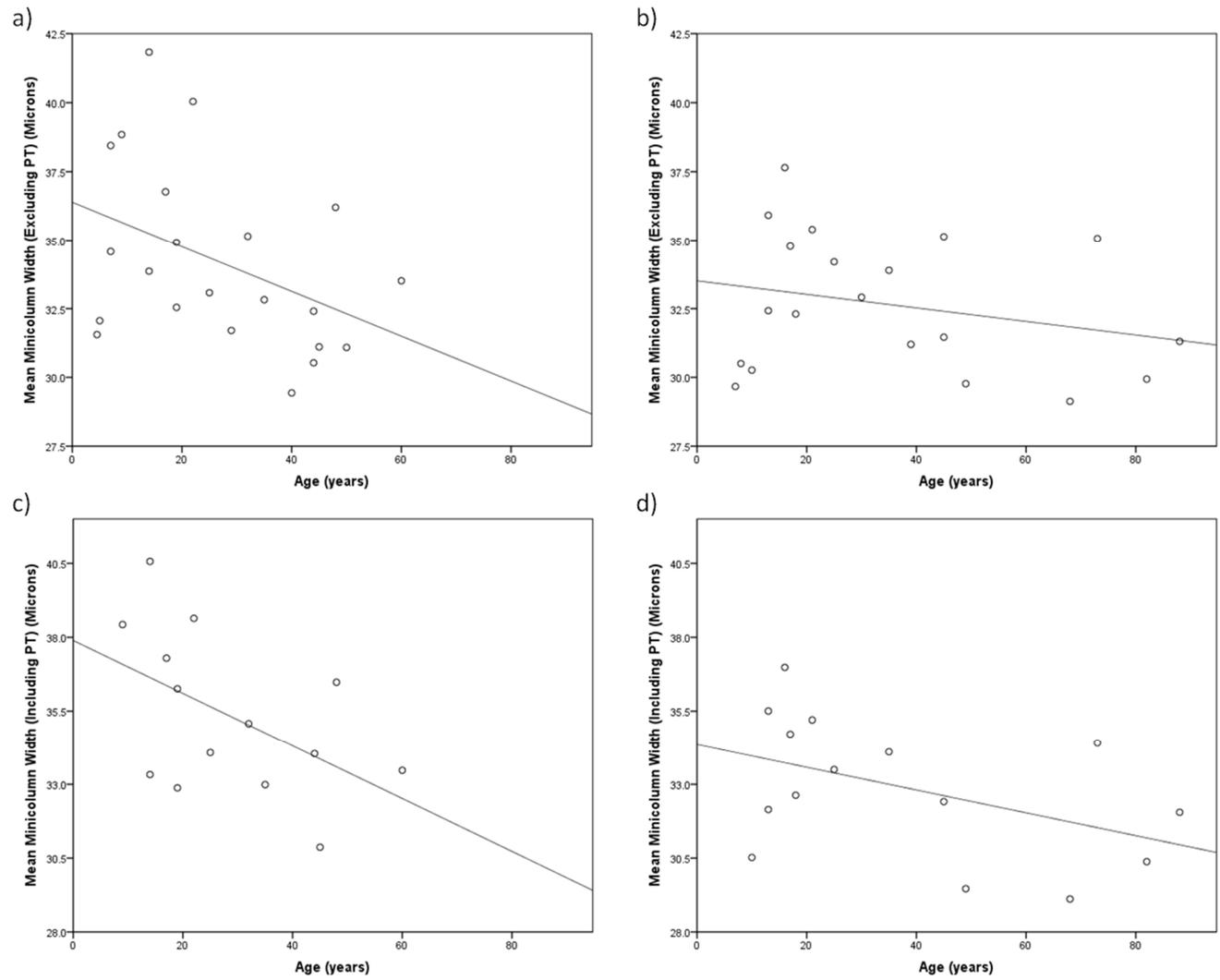


Figure 3.8. Least squares regression line for the relationship between minicolumn width and age in ASD (a, c) and controls (b, d). Graphs on the top row (a, b) represent the relationship when measurements for the PT are not included, and graphs on the bottom row (c, d) represent the relationship when measurements for the PT are included.

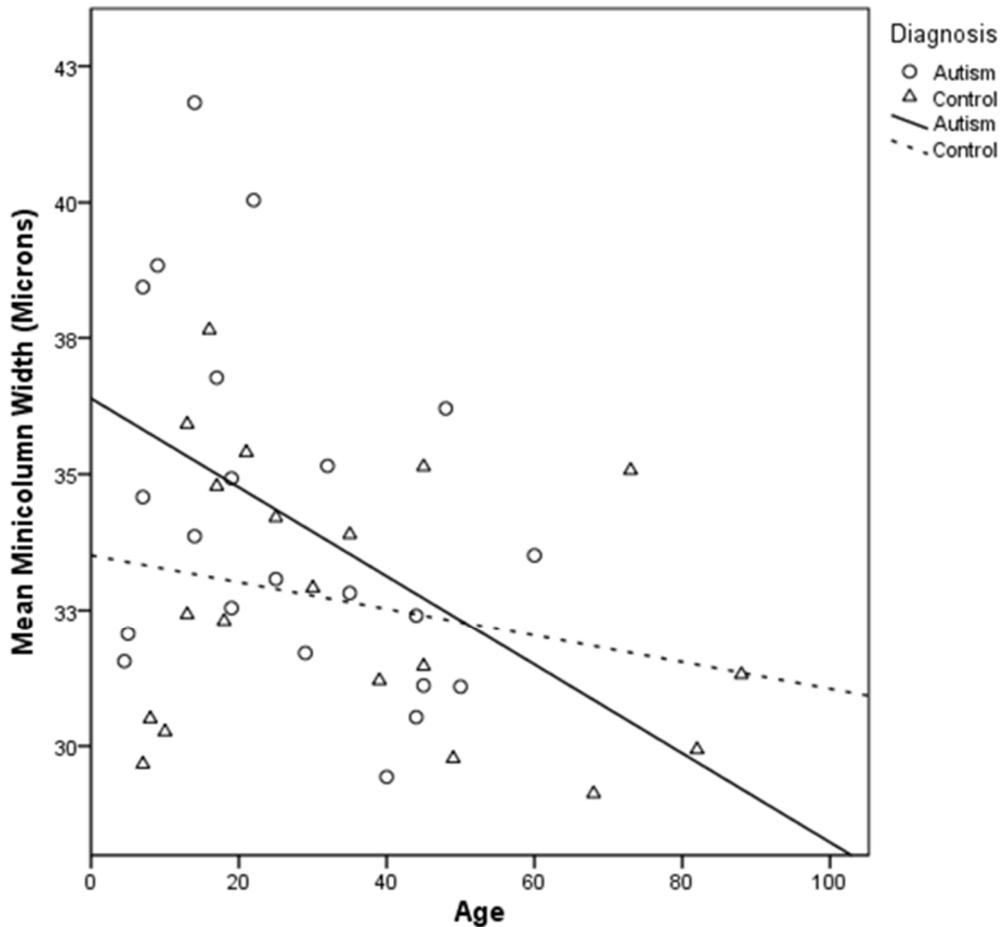


Figure 3.9. Least squares regression lines for the relationship between average minicolumn widths across BA11, BA40 and BA41 and age for ASD and control cases.

Conducting a similar analysis on relationships between total brain weight and minicolumn width in ASD revealed a significant positive relationship when PT was excluded ($r=0.624$, $p=0.007$), although this no longer reached significance when PT was included ($r=0.536$, $p=0.073$) (Figure 3.10). Investigation of the individual regions showed this relationship to be primarily driven by a significant positive relationship in ASD in BA11 only ($r=0.564$, $p=0.008$; all other $p>0.1$). No significant relationships were seen in controls (all $p>0.2$).

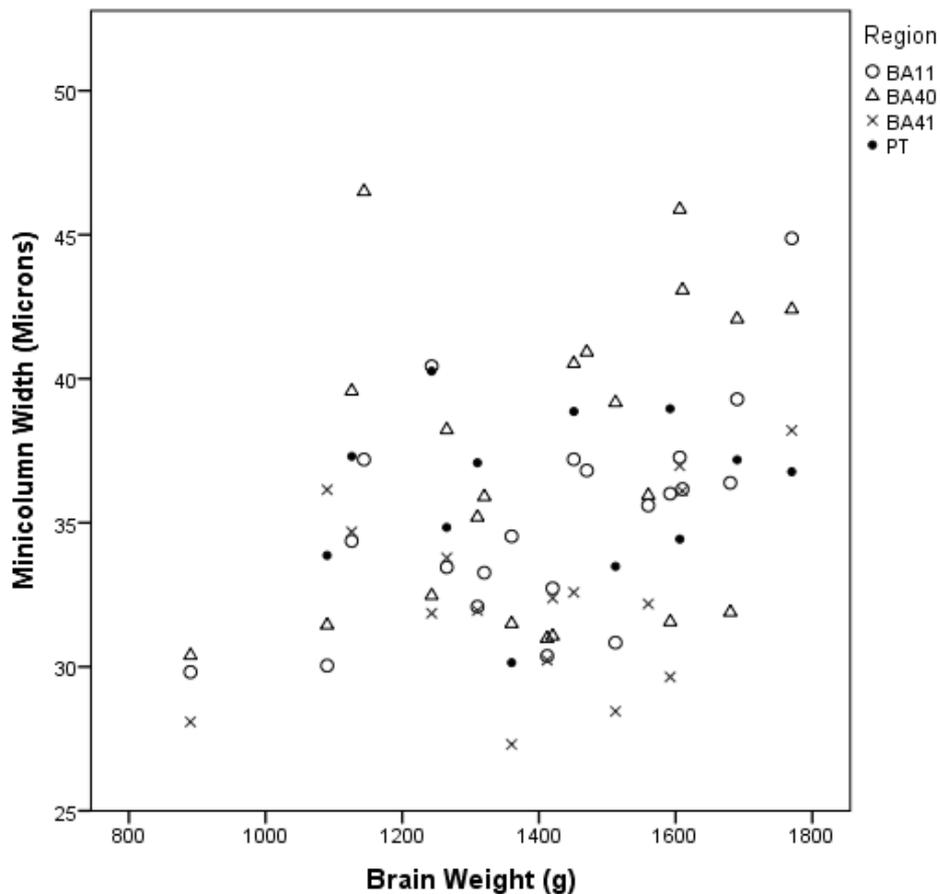


Figure 3.10. Relationship between minicolumn width and brain weight in ASD only.

Investigation of the relationship between measures of autism severity (ADI subscores) and measures of minicolumn width and overall brain weight revealed no relationships (all $p > 0.1$).

Axon Bundle Data

Regional and Diagnostic Differences

A repeated measures ANOVA with cortical region as the within subjects factor and diagnosis as the between subjects factor found a significant main effect of region on axon bundle width ($F(3,69)=7.17, p < 0.001$). Repeating this analysis for axon bundle spacing revealed no significant effects (Table 3.4).

As axon bundle data was not available for the PT for all cases (Table 3.1) the analysis was repeated with the PT excluded. No significant effects were observed for either axon bundle width or axon bundle spacing.

Post-hoc investigation of the effect of cortical region found that axon bundle widths in PT were significantly smaller than in all other regions (Figure 3.11). Inclusion of age and brain weight as covariates removed the effect of region, but this may be due to the presence of an interaction between age and cortical region ($F(3,57)=2.98, p=0.039$).

| Diagnosis | Axon Bundle Spacing (μm) | | | | Axon Bundle Width (μm) | | | |
|-----------|---------------------------------------|------|------|------|-------------------------------------|------|------|-----|
| | BA11 | BA40 | BA41 | PT | BA11 | BA40 | BA41 | PT |
| ASD | 54.1 | 56.0 | 53.3 | 54.0 | 7.1 | 7.4 | 6.8 | 5.9 |
| Controls | 52.1 | 57.0 | 55.6 | 54.7 | 7.1 | 7.4 | 6.9 | 6.2 |

Table 3.4. Average axon bundle width and spacing in each cortical region for ASD and control cases.

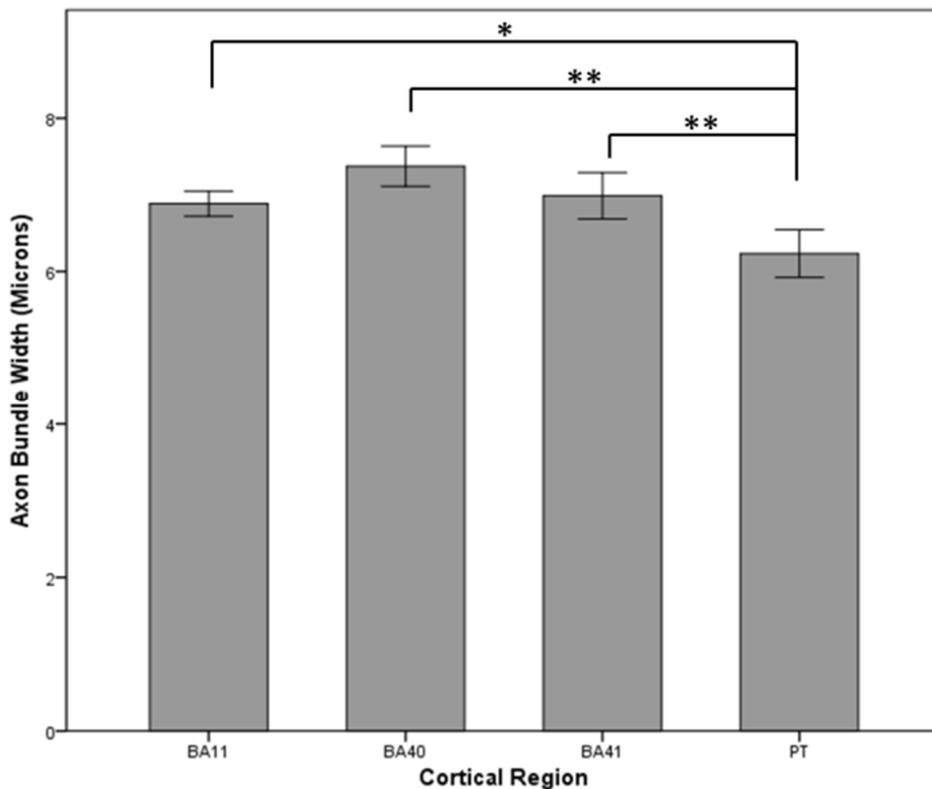


Figure 3.11. Average axon bundle widths for each cortical region. Error bars indicate the standard error of the mean.

Relationship with demographic measures

As for the minicolumn measurements, two averages were calculated for both axon bundle width and axon bundle spacing, one over BA11, BA40 and BA41, and one over BA11, BA40, BA41 and PT for those cases for which a value of PT was available. For axon bundle spacing, a positive correlation with age was observed in controls, only when PT was included ($r=0.594$, $p=0.042$; excluding PT $r=0.305$, $p=0.250$). This relationship was absent in ASD cases (excluding PT $r=0.230$, $p=0.429$; including PT $r=0.378$, $p=0.203$) (Figure 3.12a). In contrast, ASD cases showed a significant positive correlation between axon bundle width and age (excluding PT $r=0.688$, $p=0.009$; including PT $r=0.650$, $p=0.012$) which was absent in controls (excluding PT $r=0.389$, $p=0.211$; including PT $r=0.381$, $p=0.145$) (Figure 3.12b). No correlations were seen between aspects of axon bundle structure and total brain weight (all $p>0.17$).

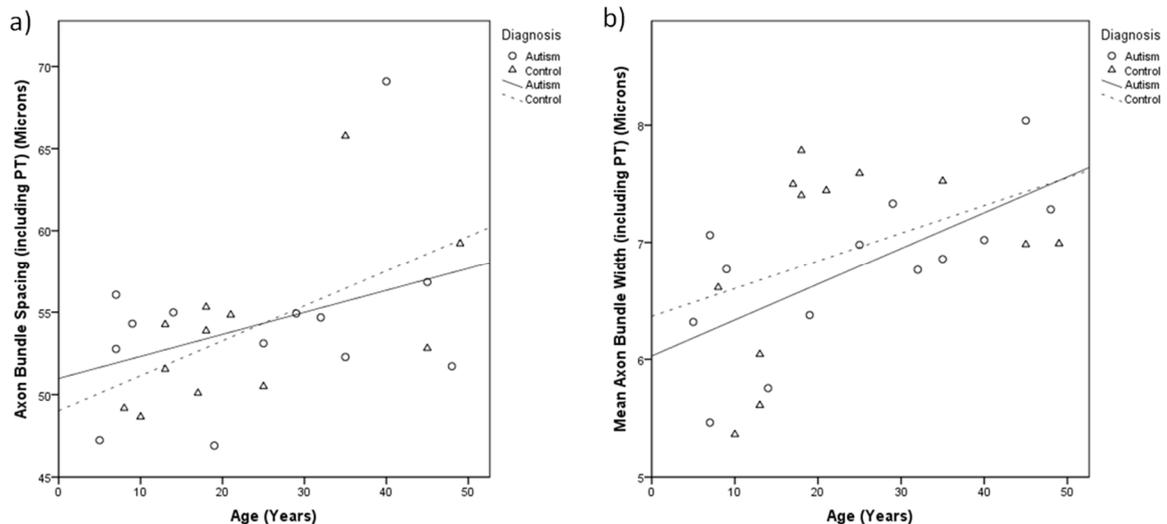


Figure 3.12. Least squares regression lines for the relationship between age and a) axon bundle spacing and b) axon bundle width.

Investigation of age effects

In order to investigate whether the diagnostic differences were more pronounced in adulthood than childhood, as suggested by Buxhoeveden et al. (2006), the cases in the present study were split into two groups: those 16 and below, and those over 16. 16 was chosen as the cut-off point as Courchesne et al. (2011b) found increased neuron numbers in children with ASD up to and including 16 years of age

A repeated measures ANOVA looking at minicolumn width, with cortical region as the within subjects factor and diagnosis and age group as the between subjects factors revealed a significant effect of region ($F(2,213,57.544)=15.911$, $p<0.001$) and diagnosis ($F(1,26)=7.856$, $p=0.009$), as well as a trend towards an effect of age group ($F(1,26)=3.807$, $p=0.062$) (Figures 3.13, 3.14). Post-hoc analysis of these effects revealed significant differences between all pairs of regions (BA11 and BA40: $p=0.019$, all other $p<0.006$; Bonferroni corrected significance level for 6 tests= 0.008) except BA11 and PT ($p=0.140$). Wider minicolumns were found in ASD cases compared to controls, and those 16 and below compared to those over 16. However, this age effect seemed to be largely driven by several elderly cases, as when those subjects aged over 60 were removed, this effect no longer reached significance.

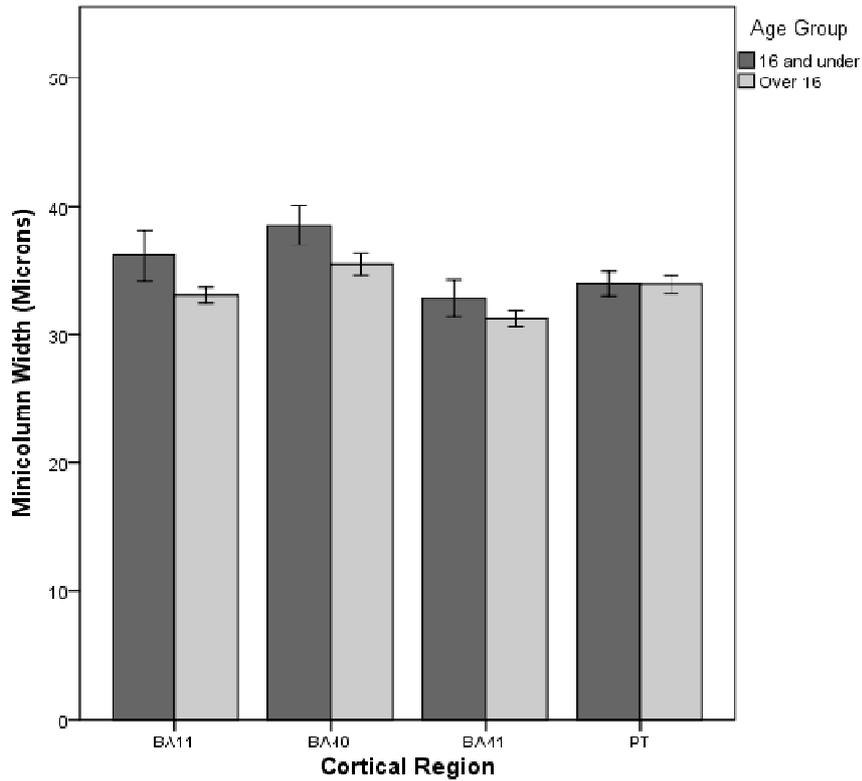


Figure 3.13. Minicolumn width across cortical regions according to age group. Error bars indicate the standard error of the mean.

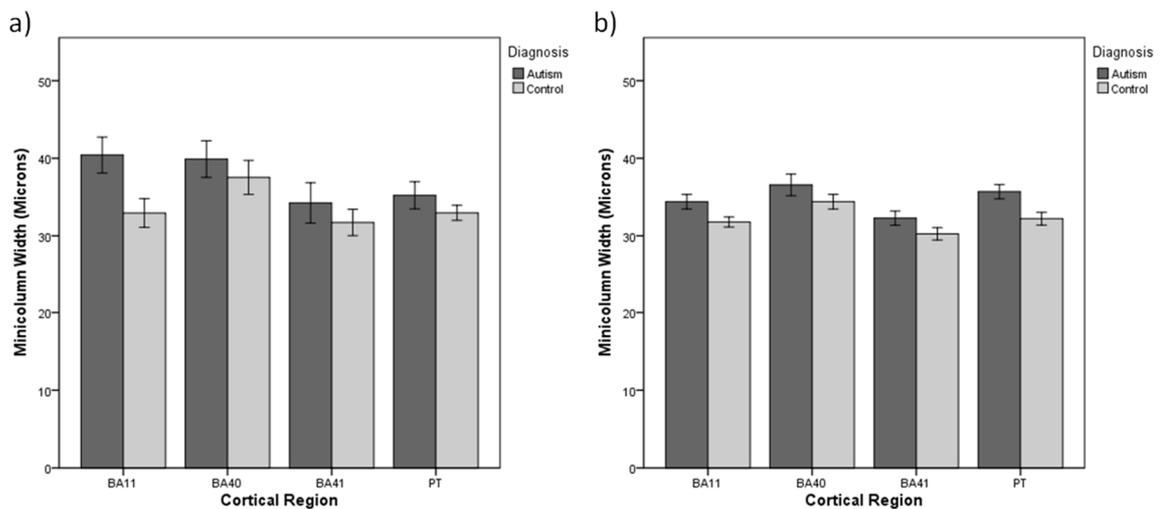


Figure 3.14 Diagnostic differences in minicolumn width by region in a) subjects aged 16 and under and b) subjects aged over 16. Error bars indicate the standard error of the mean.

Age effects on axon bundle width were investigated using a repeated measures ANOVA with cortical region as the within subjects factor and diagnosis and age group as the between subjects factor. A significant effect of both cortical region ($F(3,63)=7.66$, $p<0.001$) and age group ($F(1,21)=19.02$, $p<0.001$) along with a significant interaction

between cortical region and age group ($F(3,63)=3.63$, $p=0.018$) and a trend towards an interaction between diagnosis and age group ($F(1,21)=4.31$, $p=0.050$) were seen. No significant effects were seen for axon bundle spacing.

Post-hoc investigation of the main effects revealed significantly narrower axon bundles in PT compared to all other regions (all $p<0.006$; Bonferroni corrected significance level for 6 tests=0.008), and narrower axon bundles in those 16 and under ($p<0.001$). Post-hoc investigation of the interaction between cortical region and age revealed that although narrower axon bundles were seen in the young age group for BA40, BA41 and PT, this did not seem to be the case in BA11 (Figure 3.15).

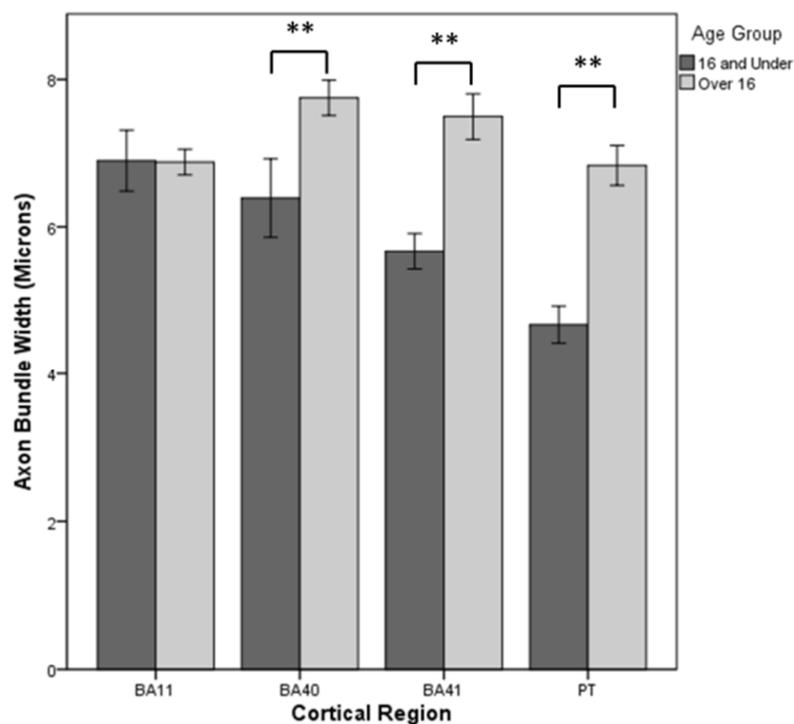


Figure 3.15. Average axon bundle width for young and old groups in each cortical region. Error bars represent the standard error of the mean.

Post-hoc investigation of the interaction between diagnosis and age group revealed an interesting pattern where axon bundle widths seem to be larger in ASD cases in the younger age group (Figure 3.16a), but larger in the control cases in the older age group (Figure 3.16b).

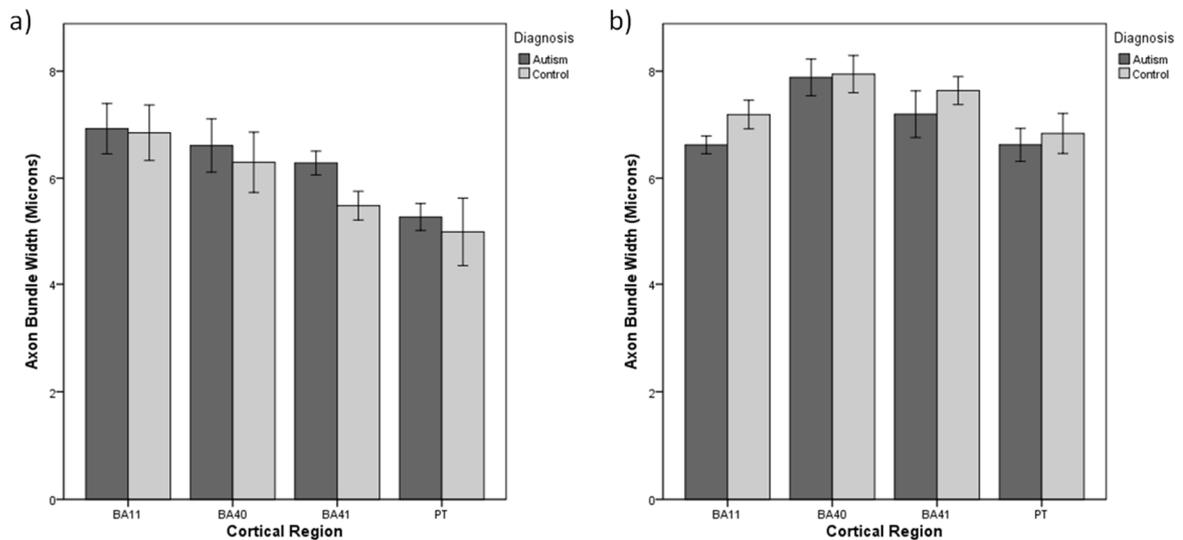


Figure 3.16. Diagnostic differences in axon bundle width for each cortical region in a) cases aged 16 and under and b) cases aged over 16. Error bars indicate the standard error of the mean.

Comparison with Previous Work

As the present study finds wider minicolumns, rather than the previously reported narrower minicolumns, in ASD, it is important to establish that this is not just an artefact of differing methodology.

Components of Minicolumn Investigated

Previous studies have reported differences in the width of the minicolumn, column core (the part of the column containing 90% of the cell bodies) and/or neuropil space (the difference between the centre-to-centre width and the width of the column core) (Casanova et al., 2002b; Casanova et al., 2006b; Casanova et al., 2010). The possibility of wider neuropil space giving rise to greater values for minicolumn width in the presence of unchanged or even decreased core values in the present study was therefore investigated. Similar results were found with a significant effect of region seen for both neuropil space ($F(3,84)=3.772$, $p=0.014$) and column core ($F(3,84)=14.421$, $p<0.001$).

Significantly wider column cores were seen in ASD ($F(1,28)=8.563$, $p=0.007$) along with a trend towards greater neuropil spacing ($F(1,28)=3.988$, $p=0.056$).

Statistical Model Used

By employing a repeated measures design with subjects closely matched for age, the results of Casanova et al (Casanova et al., 2006a; Casanova et al., 2002b; Casanova et al., 2002c; Casanova et al., 2010; Casanova et al., 2006b) may have been less affected by the seemingly different relationships between minicolumn width and age seen for ASD and control cases in the present data. In order to verify that including diagnosis as a between subjects factor, rather than using a repeated measures design did not affect the results obtained, a subset of cases for which ASD-control pairs were matched for age to within one year were identified. This resulted in 18 pairs which were analysed using a matched pairs repeated measures approach. This confirmed a statistical trend towards an effect of diagnosis ($F(1,9)=3.971$, $p=0.077$), larger values for minicolumn width were still seen in ASD cases suggesting that the differences due to analysis model are minimal. The reduced significance found in the present results when employing a matched pairs repeated measures approach may have been partly due to the reduced numbers included in the analysis.

Age

It may be possible that wider minicolumns were found in the present study due to a difference in the distribution of ages represented between studies. Indeed a greater age range of cases was included in the present study (4-88) than in previous studies finding narrower minicolumns in ASD (Table 3.5).

| Study | Number of Cases (ASD; controls) | Age Range (ASD; controls) |
|--------------------------------------|---------------------------------|---------------------------|
| Casanova et al. (2002b) ^a | 9; 9 | 5-28 ; 3-25 |
| Casanova et al. (2002c) ^a | 9; 9 | 5-28 ; 3-25 |
| Casanova et al. (2006a) ^b | 6; 6 | 4-24; 4-25 |
| Casanova et al. (2006b) ^b | 6; 6 | 4-24; 4-25 |
| Casanova et al. (2010) ^c | 7; 7 | 4-67; 4-65 |
| Buxhoeveden et al. (2006) | 2; 5 | 3-41; 2-75 |
| Present study | 28; 25 | 4.5-60; 4-88 |

Table 3.5. Age ranges of subjects included in previous studies

^a These studies use the same cases

^b These studies use the same cases

^c This study uses the 6 matched pairs from the Casanova et al. (2006a); Casanova et al. (2006b) studies with the addition of one older pair.

The data from the studies by Casanova et al. (2006a) and Casanova et al. (2006b) is available through the ATP portal (atpportal.org). Therefore, this data was analysed alongside the data from the current study to investigate age related variation in the magnitude of difference in minicolumnar measures between ASD cases and controls. In order to do so a subset of cases from the present study which could be age matched to within one year of each other were chosen. The difference in both minicolumn width and neuropil space between each matched ASD and control case was then calculated for the subset of cases from the present study as well as for the cases provided in the data from Casanova et al. (2006a) and Casanova et al. (2006b). These differences were then converted to z-scores to allow comparison between the different studies (Figures 3.18, 3.19). The data in the present study seems to show larger differences in favour of wider minicolumns in ASD at younger ages, but very little difference at older ages (Figure 3.18c). A similar pattern of results is seen in the data from Casanova et al. (2006b) (Figure 3.18b).

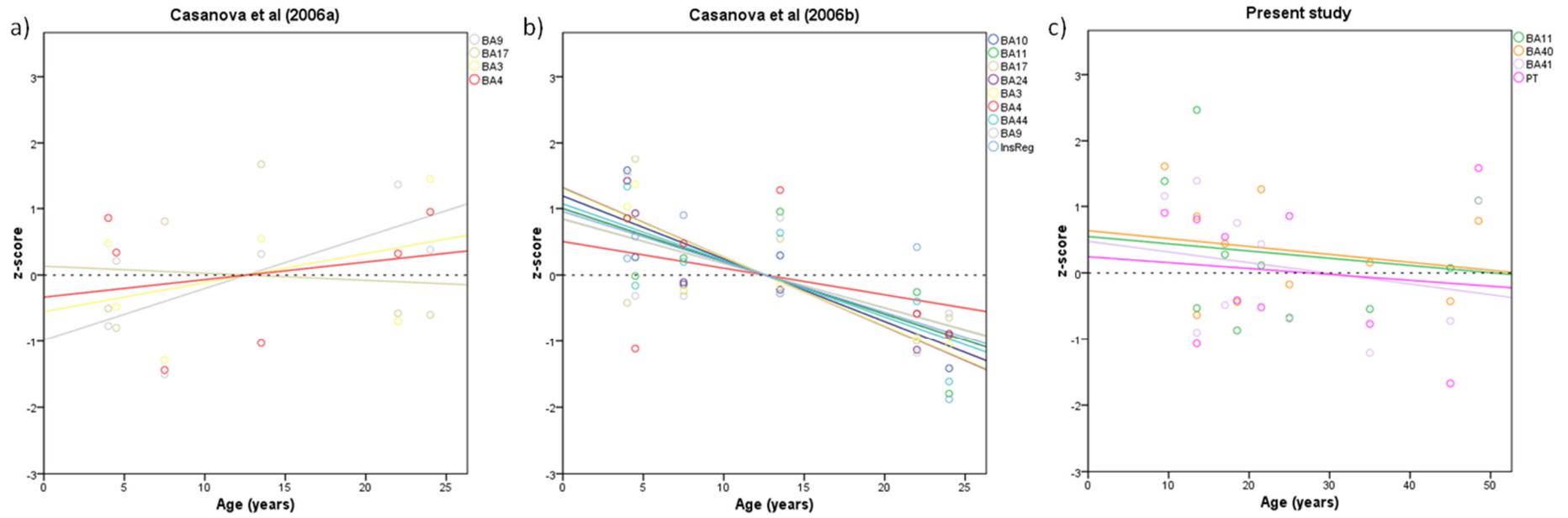


Figure 3.18. Difference in minicolumn width between ASD and control cases across the age range. Positive values indicate wider minicolumns in ASD. Data from a) Casanova et al. (2006a), b) Casanova et al. (2006b) and c) the present study

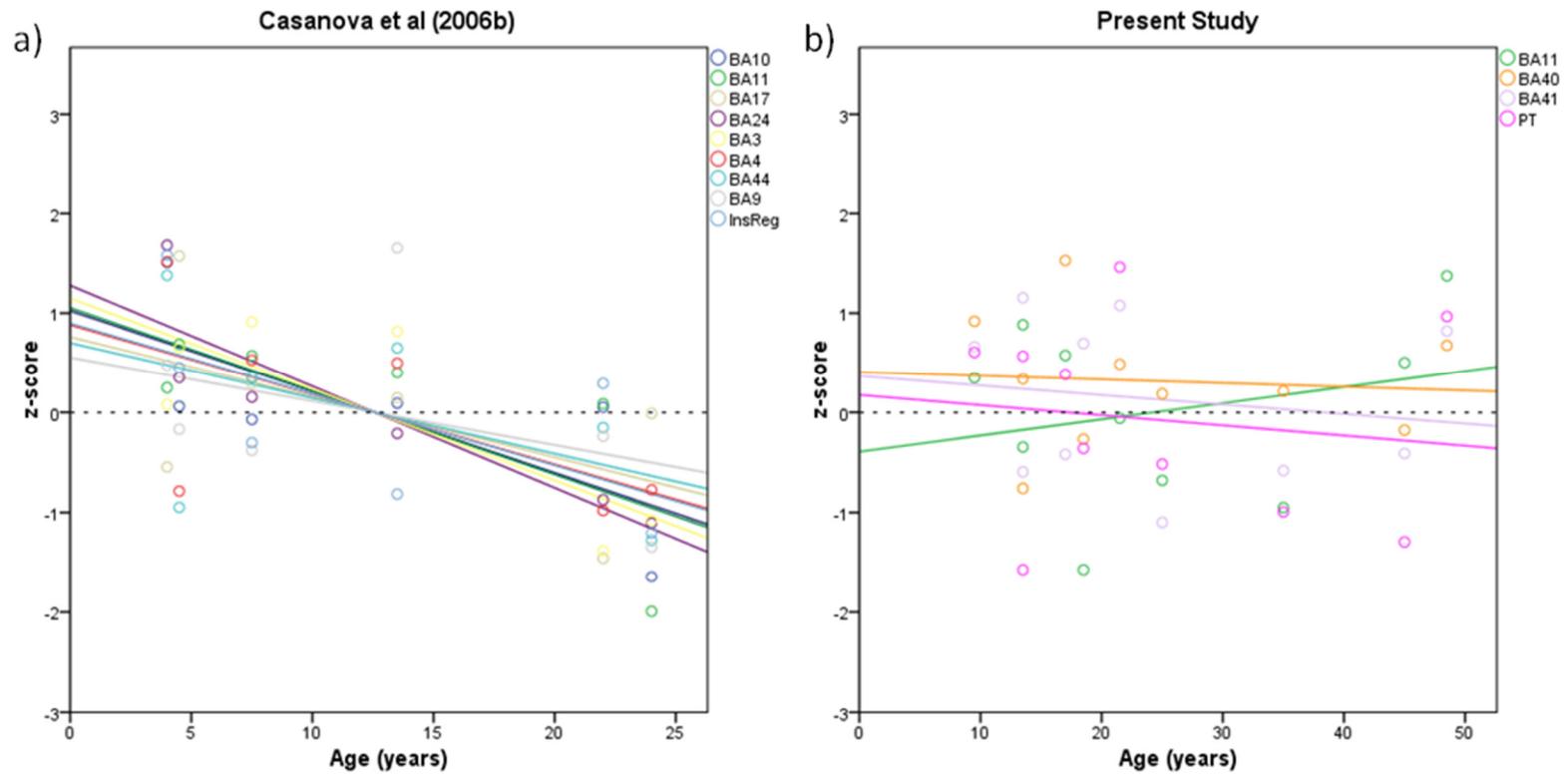


Figure 3.19. Difference in neuropil space between ASD and control cases across the age range. Positive values indicate wider neuropil space in ASD. Data from a) Casanova et al. (2006b) and b) the present study.

Investigation of how z-score changes with age revealed significant correlations between age and the z-score for difference in minicolumn width between ASD and control cases, in BA10 ($r=-0.829$, $p=0.042$), BA24 ($r=-0.914$, $p=0.011$) and BA3 ($r=-0.906$, $p=0.013$) with a trend towards a relationship in BA44 ($r=-0.746$, $p=0.088$), in the data from Casanova et al. (2006b). In the subset of data from the present study there was a trend towards a relationship between age and z-score for difference in minicolumn width in BA41 ($r=-0.445$, $p=0.084$), supporting the indication of age related variation in diagnostic differences seen in the full data set.

Choice of Laminae for Measurement

Previous studies investigating minicolumnar width have either focused on layer III (Buxhoeveden et al., 2006; Casanova et al., 2002b; Casanova et al., 2002c) or analysed minicolumn width across layers II to VI (Casanova et al., 2006a; Casanova et al., 2010; Casanova et al., 2006b). Casanova et al. (2010), in a study of ASD and control cases, found a significant effect of lamina on width of the minicolumn core and an interaction between diagnosis and lamina, suggesting that the layer in which measurements were made may affect the results, and may have a differential effect in ASD and control cases. In order to investigate whether this could have contributed to the different findings of the present study analysis of the data from Casanova et al. (2006b) was repeated, restricting the analysis to measurements from layer III only (Table 3.6). Consistent with the analysis employed in the original study a matched pairs, repeated measures ANOVA was used, with diagnosis and cortical region as within subjects factors. No main effect of diagnosis was found ($F(1,10.7)=2.134$, $p=0.204$), nor an interaction between region and diagnosis

| | | BA3 | BA4 | BA9 | BA10 | BA11 | BA17 | BA24 | BA43 | BA44 |
|------------------------------------|-----|------|------|------|------|------|------|------|------|------|
| Minicolumn Width (μm) | ASD | 21.6 | 22.9 | 21.7 | 22.4 | 22.0 | 18.8 | 23.8 | 23.5 | 23.2 |
| | CTL | 21.8 | 23.8 | 23.8 | 23.5 | 23.4 | 20.1 | 24.8 | 25.2 | 24.9 |
| Neuropil Space (μm) | ASD | 10.7 | 11.6 | 10.9 | 11.7 | 11.0 | 9.3 | 11.8 | 11.8 | 11.8 |
| | CTL | 11.0 | 12.1 | 11.9 | 11.6 | 12.0 | 9.4 | 11.8 | 12.7 | 12.6 |

Table 3.6. Average values calculated from the data from Casanova et al. (2006b) in layer III only.

($F(2.1,10.7)=0.392$, $p=0.698$), although a significant main effect of region was present ($F(2.6,10.7)=19.378$, $p<0.001$).

Tissue Processing

There were several differences in tissue preparation between studies both in terms of tissue processing and section thickness (Table 3.7).

| Study | Tissue Preparation | Section Thickness (μm) |
|---------------------------|---------------------------------------------|------------------------------------------|
| Casanova et al. (2002b) | Celloidin embedded | 35 |
| Casanova et al. (2002c) | Celloidin embedded | 35 |
| Casanova et al. (2006a) | Celloidin embedded | 200 (three hemispheres cut at 500) |
| Casanova et al. (2006b) | Celloidin embedded | 200 (three hemispheres cut at 500) |
| Buxhoeveden et al. (2006) | Two controls paraffin embedded, rest frozen | Paraffin cases = 20 Frozen cases = 80 |
| Casanova et al. (2010) | Celloidin embedded | 200 or 500 |
| Present study | Frozen | 30 |

Table 3.7. Tissue preparation methods and section thicknesses used in minicolumn studies of ASD.

As was shown in the previous chapter, section thickness is known to affect estimates of minicolumn width, with smaller values being obtained from thicker sections. In their studies using 200-500 μm sections, Casanova et al argue that the depth of field analysed was only 2.1 μm , and as this is only a small fraction of the slice thickness, differences in slice thickness would not confound the results (Casanova et al., 2006a). However, comparing the average minicolumn widths found for ASD and control cases from their 35 μm sections (Casanova et al., 2002b) with those reported from the 200-500 μm sections reveals very different numbers (Table 3.8). In addition, the values from ASD and control cases do not seem to have been affected by the same amount, with the difference in mean minicolumn widths between diagnoses expressed as a percentage of the mean minicolumn width across diagnoses falling from 12.05% in the 35 μm sections to 5.88% in the 200-500 μm sections (Table 3.8).

| Study | Section Thickness | Minicolumn Width (μm) | | | |
|---------------------------------------------------------------------|-----------------------|------------------------------------|------|-------------------------------|-----------------------------|
| | | ASD | CTL | Difference as % of mean value | ASD value as % of CTL value |
| Casanova et al. (2002b) | 35 μm | 46.8 | 52.8 | 12.1 | 88.6 |
| Casanova et al. (2006a) | 200-500 μm | 25.7 | 27.2 | 5.6 | 94.6 |
| 200-500 μm value as percentage of 35 μm value | | 54.9 | 51.5 | | |

Table 3.8. Comparison of average minicolumn widths found in two studies by Casanova's group (Casanova et al., 2006a; Casanova et al., 2002b).

Cortical Region Investigated

As BA11 is the only region to have been looked at both in the present and previous studies, a more in-depth analysis of the data from Casanova et al. (2006b) for this region was conducted. Although minicolumn width was not observed to differ significantly between ASD and control cases in BA11, values were on average lower in ASD compared to controls (22.0 μm vs. 23.4 μm). As the original analysis involved treating the cases as matched pairs (Casanova et al., 2006b), the differences between pairs for BA11 were also examined, and although two pairs showed higher values in the ASD cases, the remaining pairs showed higher values in the control cases (although pair 3 seems to be an outlier) (Figure 3.20).

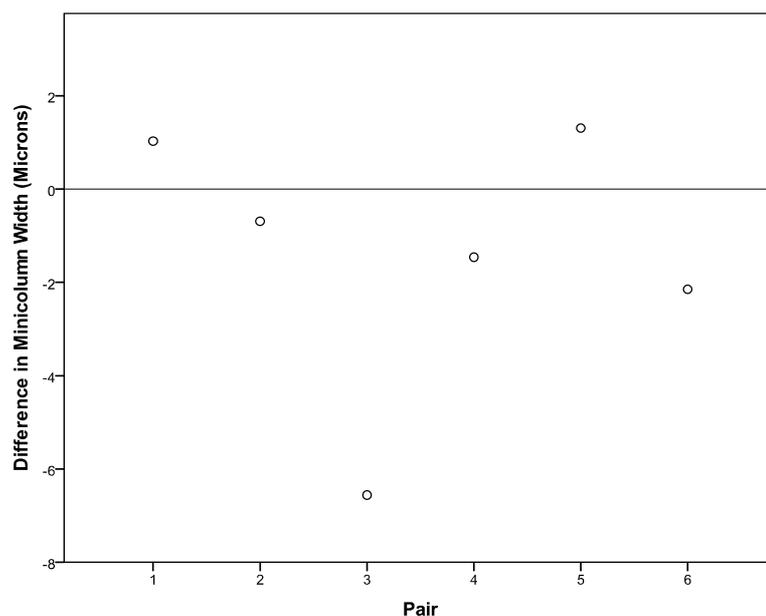


Figure 3.20. Differences in minicolumn width in BA11 for the matched pairs studied by Casanova et al. (2006b). Positive values indicate wider minicolumns in ASD.

| Study | BA3 | | BA4 | | BA11 | | BA9 | | BA10 | | BA44 | | BA17 | | BA40 | | BA41 | | PT | | BA43 | | BA24 | | |
|----------------------------------------------------|------|------|------|------|-------|-------------------|--------------------------------------|------|-------|------|------|------|------|------|------|------|-------|------|-------|------|------|------|------|-------------------|------|
| | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | ASD | CTL | |
| Minicolumn Width (μm) | | | | | | | | | | | | | | | | | | | | | | | | | |
| Casanova et al. (2006a) ^b | 25.8 | 27.0 | 27.5 | 28.2 | | | 26.5 | 29.4 | | | | | 23.0 | 24.1 | | | | | | | | | | | |
| Buxhoeveden et al. (2006) ^c | | | | | 40.2 | 46.9 ^f | ASD = 42.0 ; CTL = 47.3 ^d | | | | | 31.5 | 32.7 | | | | | | | | | | 49.3 | 50.4 ^g | |
| Postmortem scanning | | | | | | | 38.9* | 32.8 | | | | | 24.1 | 24.5 | | | | | | | | | | | |
| Present study | | | | | 34.8* | 32.5 | | | | | | | | | 36.2 | 35.1 | 32.1 | 30.4 | 35.6* | 32.6 | | | | | |
| Core Width (μm) | | | | | | | | | | | | | | | | | | | | | | | | | |
| Casanova et al. (2010) ^{a, b} | 9.5 | 12.5 | 9.5 | 12.5 | 9 | 11.5 | 8.5 | 12 | 9.5 | 12 | 8 | 13 | 9.5 | 12 | | | | | | | | 9 | 13 | 10 | 11.5 |
| Post-mortem scanning | | | | | | | 30.0 | 26.8 | | | | | 21.9 | 21.7 | | | | | | | | | | | |
| Present study | | | | | 27.1 | 25.7 | | | | | | | | | 28.2 | 27.4 | 25.8* | 24.6 | 27.7 | 26.5 | | | | | |
| Neuropil Space (μm) | | | | | | | | | | | | | | | | | | | | | | | | | |
| Casanova et al. (2006b) | 11.1 | 11.1 | 11.9 | 11.2 | 11.5 | 12.0 | 11.2 | 11.9 | 11.7* | 11.2 | 12.0 | 12.7 | 9.1 | 9.8 | | | | | | | | 12.1 | 12.3 | 12.4* | 11.0 |
| Postmortem scanning | | | | | | | 8.9 | 5.9 | | | | | 2.1 | 2.8 | | | | | | | | | | | |
| Present study | | | | | 7.7 | 6.7 | | | | | | | | | 8.0 | 7.7 | 6.3 | 5.8 | 7.9* | 6.0 | | | | | |

Table 3.9. Average values for minicolumn width, core width and neuropil space in all cortical regions examined to date. * indicates statistically significant differences between ASD and control groups ($p < 0.05$).

^a Values calculated from the figure provided in the paper.

^b No pairwise comparisons reported in the paper to assess statistical differences between diagnoses within cortical regions.

^c No pairwise comparisons reported. A significant effect of diagnosis was seen for frontal regions but not BA17.

^d Value provided for dorsal frontal cortex

^f Value provided for orbitalfrontal cortex

^g Value provided for mesial frontal cortex

Discussion

In contrast to previous findings (Buxhoeveden et al., 2006; Casanova et al., 2006a; Casanova et al., 2002b; Casanova et al., 2006b), the current study finds wider minicolumns in ASD. This does not appear to be restricted to higher order association areas which has important implications for understanding how processing may be altered in ASD. This is the largest study to date and includes cases across a wide age range, providing some evidence of an altered developmental trajectory in ASD.

Diagnostic differences

It is not clear why the results of the present study differ from those of several previous studies, although the other studies mostly investigated different brain regions on substantially smaller sample sizes (see section on study comparisons below). Yet the present findings are not incompatible with the broader literature. A number of studies and theories (Happé and Frith (2006); Mottron et al. (2006) and Plaisted (2001)) support the idea of a bias towards a local, rather than global, processing style in ASD. This suggests that when presented with a stimulus the features of that stimulus are processed individually rather than holistically as typically seen in controls. Wider spacing of minicolumns, as in the present study, has been shown to result in less overlap of the dendritic trees associated with the constituent neurons (Seldon, 1981a). This in turn has been suggested to result in less co-activation of neighbouring minicolumns allowing them to function more independently and so facilitate the processing of individual features as seen in ASD (Chance et al., 2012; Harasty et al., 2003). In contrast, narrower minicolumns are more likely to be co-activated and so facilitate holistic processing of stimuli. There is evidence from the neuroimaging literature to support this, magnetic resonance imaging studies have reported alterations in grey matter brain chemistry which

are consistent with a reduced number or density of dendrites (Friedman et al., 2006; Friedman et al., 2003), which may reflect the reduction in dendritic density resulting from wider spacing of the same amount of dendrites. It would be interesting for future work to investigate this further in ASD to obtain histological evidence of whether there are less overlapping dendritic trees resulting from the wider spacing of minicolumns.

Due in part to genetic findings, and also to the explanatory power of such a model for other aspects of autism, increasing attention is being paid to models suggesting there is disruption of the excitation-inhibition ratio in ASD (Pizzarelli and Cherubini, 2011; Polleux and Lauder, 2004; Rubenstein and Merzenich, 2003). An increase in the ratio of excitation compared to inhibition has been proposed to explain a number of features of ASD (Rubenstein and Merzenich, 2003) although, perhaps most convincingly, it provides an explanation for the high co-morbidity between ASD and epilepsy (Polleux and Lauder, 2004). Importantly for the findings of the current study, computational models investigating the development of cortical minicolumns suggest that decreases in inhibitory strength will bias towards the formation of wider minicolumns (Gustafsson, 1997; Gustafsson, 2004).

Although findings concerning neuronal densities in ASD have been mixed (Courchesne et al., 2011b; Oblak et al., 2011; van Kooten et al., 2008), these have largely investigated different cortical regions from those examined in the present study (although one magnetic resonance spectroscopy study reported lower levels of N-acetylaspartate (NAA) in BA41 in ASD, which they argued was consistent with a low density of neurons in this area (Hisaoka et al., 2001)). In addition, studies examining neuronal density do not discriminate between the radial and tangential spacing of the cells. If we consider a minicolumn to be defined as one cell wide (Casanova and Switala, 2005; Peters, 2010; Seldon, 1981a) and measuring minicolumn width by centre-to-centre spacing of the cell

bodies (and so including the neuropil space, as was done in the present study), an increase in minicolumn width would be expected to result in a decrease in cell density in the horizontal direction (i.e. the direction parallel to the cortical surface). However, measures of minicolumn width do not indicate whether neuronal density is changed in the radial (i.e. vertical) direction. It is possible that even in the presence of decreased horizontal density, this could be counterbalanced, or even outweighed by increases in radial neuronal density. It has been shown that the increase in horizontal spacing of cells in lower layers of the cortex (IV-VI) in concave regions (i.e. at the fundi of sulci) is accompanied by a thinning of these lower layers, and so a reduction in the vertical spacing of cells, resulting in very little change in overall cell density (Chance et al., 2004). Therefore, changes in minicolumn width may not necessarily be reflected by changes in neuronal density.

This is the first study to examine aspects of axon bundle organisation in the cortex in ASD, however these axon bundles ultimately enter the white matter below the cortex, which has been investigated in ASD in several studies (Herbert et al., 2004; Zikopoulos and Barbas, 2010). Although it is perhaps surprising that we did not find any diagnostic differences in axon bundle spacing this is consistent with data finding no difference (in ASD compared to controls) in axon density in the white matter immediately underlying the cortex (Zikopoulos and Barbas, 2010). An additional, future study, preferably with electron microscopy, would be required to determine which parameters of the axon bundles were altered to give rise to narrower axon bundles (e.g. changes in axonal density, changes in the number of axons or changes in the size of individual axons, changes in myelination). However, it is interesting that Zikopoulos and Barbas (2010) found region specific reductions in the number of large axons and an increase in the number of small axons in adult ASD cases. This suggests that there may be a shift

towards smaller axons in ASD which could in turn lead to narrower axonal bundles. Smaller axons mediate short range connections (Zikopoulos and Barbas, 2013) and a bias towards smaller axons is compatible with evidence suggesting a reduction in long range connectivity while short range connectivity is preserved or even increased (Courchesne and Pierce, 2005). It would be interesting for future work to investigate whether this is also true in the white matter underlying the regions examined in the present study, and whether reduced axon diameter is seen in narrower axon bundles. In contrast, the finding of wider axon bundles in ASD in the younger age group suggests either enlargement of individual axons or increased numbers of axons. Increased numbers of small axons mediating connections between neighbouring areas would lead to an increase in the volume of white matter immediately underlying the cortex, which is the part of the white matter which has been found to show enlargements at early ages (Herbert et al., 2004).

Age

The findings of the present study suggest an effect of age on the difference in minicolumn width between ASD and control cases. A significant negative correlation is seen between minicolumn width and age for ASD subjects but not in controls (Figure 3.19). Although there is only one ASD case that exceeds the age of predicted intersection of the best fit lines, there seems to be a trend for minicolumn widths in ASD and controls to become more similar with age.

In addition, when the cases are split according to those over 16 and those 16 and below, differences in minicolumn width appear more pronounced in the younger age group (Figure 3.13), although this does not reach significance in this study. Similarly, when a subset of cases was chosen to conduct a matched pairs analysis, the differences in minicolumn width again seemed more pronounced at younger ages (Figure 3.18c).

Although a similar pattern was not observed in the data from Casanova's 2006a study (Casanova et al., 2006a), it was seen in their 2006b study (Casanova et al., 2006b) (Figure 3.18a, and 3.18b) this could be due to the regions investigated as the present study has only BA11 in common with those studies.

This pattern of larger differences at younger ages seems to fit with the whole brain volume data in ASD, which shows larger brains in ASD at young ages but that this difference disappears in adulthood (Courchesne et al., 2011a) (Figure 3.17).

However, in contrast to the total brain volume data where diagnostic differences disappear largely due to controls 'catching up', the diagnostic differences in minicolumn width appear to reduce through reduction of minicolumnar width with age in ASD cases only. Increased minicolumnar width could contribute to the increases seen in grey matter at early ages, particularly those seen in total surface area (Mak-Fan et al., 2012) (although there is clearly also a large white matter contribution to the total brain volume increases seen (Herbert et al., 2004)). One recent study found larger brain surface areas in young children with ASD which decreased with age until it reached a similar value to that seen in controls at around age 9 (Mak-Fan et al., 2012). Although we still see differences in minicolumnar width at later ages than this, Mak-Fan et al. (2012) found that some regions were still showing increases in surface area in ASD at later ages. The results were only reported at a lobar level, meaning we cannot tell whether the specific age range over which surface area enlargements are seen corresponds to that over which wider minicolumns are seen in certain regions. However, the pattern of higher values in ASD which then decrease with age, becoming more similar to the values seen in controls, is similar to the pattern we observe in the minicolumn data. Increased minicolumn width in ASD may bias towards a local processing style at early ages, such that, although

diagnostic differences in total brain volume, and later minicolumn width, diminish with age, the bias in processing style has already been established.

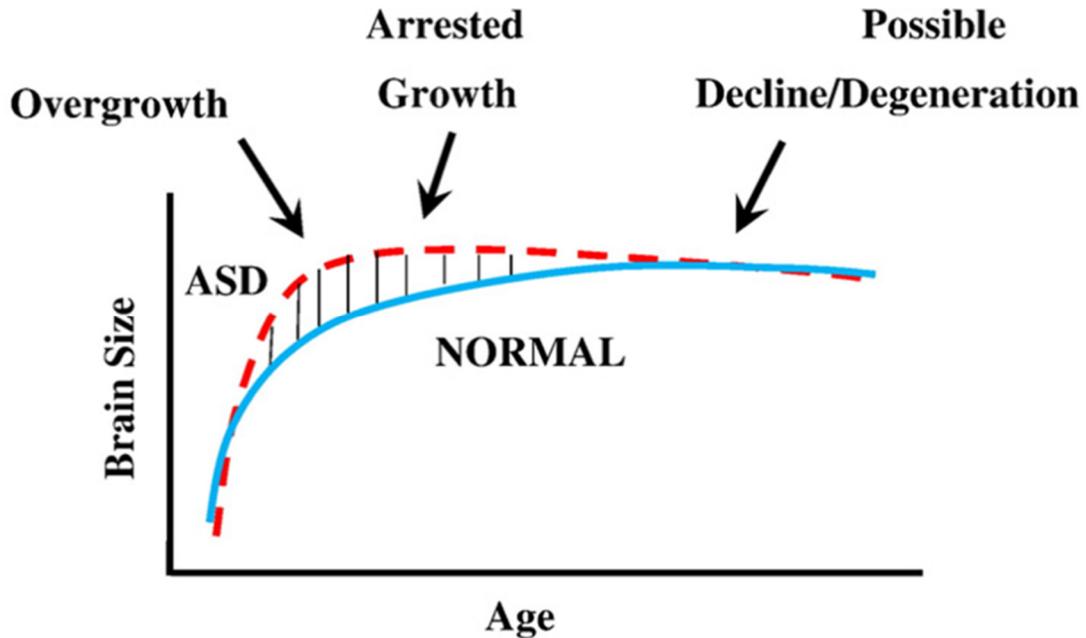


Figure 3.17. Relationship between age and total brain volume (from Courchesne et al. (2011a))

Regional differences

The present study found regional differences in both minicolumn width and axon bundle width, with BA40 showing the widest minicolumns and axon bundles and BA41 showing the narrowest. This is consistent with previous reports that minicolumn width relates to cortical hierarchy, with the narrowest minicolumns being seen in primary sensory regions (in this case BA41) and the widest minicolumns being seen in heteromodal sensory regions (of which BA40 is an example) (Chance, 2006). Although it is well established that cortical regions differ in their myeloarchitecture as well as cytoarchitecture (DeFelipe, 2005), the former has generally been overlooked in the literature and so regional differences are not well characterised. The one study that has investigated axon bundle width in BA41 found slightly higher values than those found in the present study (Seldon, 1981b), although that may reflect differences in tissue processing (celloidin

embedded in Seldon (1981b) vs. frozen tissue in the present study) or the wider age range included in the present study (5-50 years in the present study vs. 74-89 years in Seldon). It is consistent that the areas showing the widest minicolumns also showed the widest axon bundles and vice versa. It is not possible using this current technique to separate out the different contributions of increases in individual axon width, number of axons and axon density to increases in axon bundle width. However, it is possible that if there were an increase in individual axon width this would be accompanied by an increase in neuronal size (Vinters and Kleinschmidt-DeMasters, 2008) both of which would therefore contribute to an increase in minicolumn width.

Given recent findings from both genetic and imaging studies suggesting less differentiation between cortical regions in ASD (Voineagu et al 2011, Ziats and Rennert 2013, Minshew and Keller 2010) it is notable that this does not seem to be reflected in the minicolumn data. The present study found a significant main effect of region, but no interaction between region and diagnosis, suggesting the pattern of regional differences is not different between ASD cases and controls, and regional differentiation is preserved in ASD. Although the previous studies compared some different regions to those investigated here (Voineagu et al (2011) compared BA9 with BA41/42 and Ziats and Rennert (2013) compared prefrontal cortex with cerebellum) previous minicolumn studies looking at these regions also found no interaction between cortical region and diagnosis (Casanova et al., 2006a; Casanova et al., 2002b). However, previous findings have found the attenuation of gene expression is only seen between some areas and not others (Ginsberg et al., 2013; Voineagu et al., 2011; Ziats and Rennert, 2013), suggesting that attenuation of regional differences in minicolumn width may occur in ASD but may be more pronounced in regions of cortex not covered by the present study.

Although the present study found a significant effect of region for both ASD and control cases considered individually, post-hoc testing revealed fewer regional differences in ASD than in controls, suggesting that cortical differentiation is not absent, but may be attenuated. The present study considered this across the lifespan, however, and did not investigate this at different age points. It is known that controls show a reduction in cortical differentiation with age, with higher order association regions showing a reduction in minicolumnar width after 65 years of age, which is not seen in primary sensory regions (Chance et al., 2006). Additionally, subjects with ASD are known to show an altered developmental trajectory, with a possibly accelerated onset of degeneration (Courchesne et al., 2011a). Therefore, given the present data it is not possible to distinguish between a scenario where ASD is characterised by reduced cortical differentiation from childhood, and one where ASD is characterised by an earlier onset of reduction in minicolumn width, resulting in apparently reduced cortical differentiation when average minicolumn width is considered across the age range. Visual inspection of the data in Figure 3.13, however, seems to support the latter explanation, although future studies should investigate this formally.

Although the difference in minicolumn width is not significant for all regions it does not follow the pattern of differences being present in 'higher order' associative regions rather than primary sensory regions as suggested by Buxhoeveden et al. (2006). Although not reaching statistical significance, BA41, a primary sensory region, showed a trend towards wider minicolumns in the present study. Conversely, BA40, a heteromodal association area, does not show a significant difference in minicolumn width between ASD cases and controls.

Comparison with Previous Work

Components of Minicolumn Investigated

Minicolumnar abnormalities in ASD were originally reported to affect both the centre-to-centre minicolumn width and the neuropil component of that distance specifically (Casanova et al., 2002b). Neuropil space is calculated by finding the difference between the centre-to-centre width and the width of the column core. The column core is the part of the column that contains 90% of the cell bodies (Casanova et al., 2002b). Although the original study reported differences in both minicolumn width and neuropil space (Casanova et al., 2002b), other studies have reported differences in just width of the column core (Casanova et al., 2010) or neuropil space (Casanova et al., 2006b). Although most findings have been of reductions in width of the minicolumn components, Casanova et al. (2006b) found the neuropil space component was significantly **greater** in ASD than control cases in BA10 and BA24. The present study found wider column cores and greater neuropil spacing in ASD, suggesting the difference in the present study does not reflect the decision to look specifically at one particular aspect of the minicolumn.

Age

Differences in minicolumn width have previously been reported to be more pronounced in adults than children (Buxhoeveden et al., 2006). In fact, in a study of one ASD and one control child Buxhoeveden et al. (2006) reported wider minicolumns in the mesial frontal cortex in ASD (45.7 μ m vs. 42.5 μ m in the control case).

In order to investigate the effect of age on the direction of difference found the results from the present study were compared with the results obtained by Casanova et al. (2006a) and Casanova et al. (2006b). Interestingly, the same direction of relationship is

seen in both the data from Casanova et al. (2006b) and the data from the present study, suggesting wider minicolumns in ASD at young ages, but that this difference reduces with age. The difference between the studies therefore seems to result from the fact that Casanova et al. (2006b) find wider minicolumns in controls by around age 20, whereas the present study finds little difference between groups at that age. This may in part reflect differences in cortical regions studied, as the strongest relationships between age and z-score for the difference in minicolumn width in the Casanova et al. (2006b) data are seen in regions not investigated in the present study (BA10, BA24 and BA3). In addition, as can be seen from the data from the present study, there is considerable variation between cases, and so by including fewer cases the Casanova et al. (2006b) data may be skewed towards finding an earlier age at which the relationship changes from wider minicolumns in ASD to wider minicolumns in controls.

Choice of Laminae for Measurement

The present study measured minicolumn width in layer III, as minicolumns are clearest there. Although analysis also focused on layer III in some of the first studies to report reduced minicolumnar width (Buxhoeveden et al., 2006; Casanova et al., 2002b; Casanova et al., 2002c), some of the later studies analysed minicolumn width across layers II to VI (Casanova et al., 2006a; Casanova et al., 2010; Casanova et al., 2006b). Casanova et al. (2010), in a study of ASD and control cases, found a significant effect of lamina on width of the minicolumn core and an interaction between diagnosis and lamina, and, although not statistically significant, there was a tendency for minicolumn width to increase with cortical depth in the data from Casanova et al. (2006b). Although no interaction between layer and diagnosis was found for minicolumn width in the Casanova et al. (2006b) data, analysis of the data obtained through the ATP portal was

repeated restricting the analysis to measurements from layer III only. As originally reported in the paper, no main effect of diagnosis was found, but average measures of both minicolumn width and neuropil space were smaller in ASD than control cases (with the exception of neuropil space in BA10 and BA24) (Table 3.6). This suggests that although differences in which layer measurements are taken from may affect comparability between studies, it is not responsible for the discrepancy between the present findings and those of previous studies.

Tissue Processing

There were several differences in tissue preparation between studies (Table 3.7), something which has previously been shown to affect shrinkage of tissue (Carlo and Stevens, 2011; Dobrin, 1996; Gardella et al., 2003) and so may affect estimates of minicolumnar width. Studies by Casanova's group used celloidin embedding which, like the freezing method used in the present study, has been shown to result in relatively little shrinkage (Carlo and Stevens, 2011; Dorph-Petersen et al., 2001; Gardella et al., 2003). In contrast, paraffin embedding has been shown to result in considerable shrinkage, particularly in the z dimension (Gardella et al., 2003; Nielsen et al., 1995). The differing fixation methods used in the study by Buxhoeveden et al. (2006) (Table 3.7) may have implications for comparisons within the study, though the results between studies using frozen or celloidin embedded tissue should not differ greatly, and there is certainly no reason to expect ASD and control tissue to be differentially affected.

Although the different preparation methods should have little impact on the comparability of the present results with those of previous studies, the differences in section thickness may have more of an impact. As was shown in the previous chapter, section thickness is known to affect estimates of minicolumn width, with smaller values

being obtained from thicker sections. This was also found when looking at the data from Casanova et al. (2002b) and Casanova et al. (2006a). In addition, the values from ASD and control cases do not seem to have been affected by the same amount (Table 3.8). Although these two studies looked at different cortical regions (Casanova et al. (2002b) looked at BA21, BA22 and BA9 while Casanova et al. (2006a) looked at BA3, BA4, BA9 and BA17), this still raises the possibility that ASD and control tissue may be differentially affected by either section thickness or another aspect of the analysis procedure. Although this would need to be investigated further to determine whether this is the case, it could potentially reflect underlying differences in minicolumn width between ASD and controls.

Cortical Region Investigated

As can be seen from Table 3.9, there is considerable variability in the cortical regions investigated between the studies, with only BA11 having been looked at both in the present and previous studies. Although Buxhoeveden et al. (2006) reported values of minicolumn width, it is unclear exactly which area of orbital frontal cortex they looked at and so whether it would be expected to be comparable with the values obtained from BA11. In contrast, studies from Casanova et al (Casanova et al., 2010; Casanova et al., 2006b) look at BA11 specifically, and, although they do not report measures of minicolumn width in those papers, these can be calculated from the raw data. Analysis of the data from Casanova et al. (2006b) failed to reveal an entirely consistent pattern, although overall minicolumns seemed to be narrower in ASD.

Although this does not help to shed light on the present finding of wider minicolumns in ASD in BA11, the fact that no previous studies have compared minicolumn width

between ASD and control cases in BA40, BA41 or PT makes it difficult to say whether there is a discrepancy between what is found in the present study and previous studies, or whether ASD might in fact be characterised by areas of both increased and decreased minicolumn width.

Conclusion

This is the largest study to date to investigate minicolumn widths in ASD and finds that contrary to previous reports, minicolumn width is increased in some areas of the brain. In addition both primary sensory and higher order associative areas seem to be affected, though there does seem to be evidence for reduced cortical differentiation which may reflect an altered developmental trajectory. Although this study was not formally able to address the issue of age, the results suggested that the differences between ASD and control cases are more pronounced at earlier ages, and this is something future work should aim to investigate more fully.

The present finding of wider minicolumns has important implications for understanding the neural basis of ASD, as this may be what is driving the bias towards a more local, feature-oriented processing style. In order to further validate this conclusion, future work should investigate the extent of overlap of dendritic trees in these areas in ASD.

Chapter 4:

Post-mortem Scanning - Multiple Sclerosis

Although histological examination of post-mortem tissue forms the gold standard for investigating the microstructure of the brain, this is a destructive process and is severely limited by the availability of tissue. This scarcity of post-mortem tissue is particularly true of ASD and is compounded by the fact that ASD is identified on the basis of behavioural criteria, and so cannot be diagnosed before these emerge, resulting in no tissue being available for a pre-symptomatic stage. The work described in this and the following chapter represent the initial stages of the development, and validation, of a method for probing the microstructure of the cortex using magnetic resonance imaging (MRI), with the ultimate aim of this method being adapted for in-vivo use. It is suggested that the columnar organisation, which was demonstrated to be altered in ASD in the previous chapter (Chapter 3), may contribute towards the directional diffusion observed in the cortex, and the present work aims to test this quantitatively, and investigate whether cortical diffusivity demonstrates the diagnostic and regional sensitivity shown by histological measures. Successful adaptation of this method for in-vivo use would allow investigation of cortical microstructure in larger samples without the confounds inherent in post-mortem studies (e.g. post-mortem interval), as well as opening up the possibility of longitudinal studies and monitoring of individuals at high risk of developing ASD in order to identify possible biomarkers.

Due to the scarcity of post-mortem ASD brains, the availability of previously acquired scans from post-mortem multiple sclerosis brains and the possibility that the demyelination occurring in multiple sclerosis may provide a wider range of both

histological and MRI values making it easier to detect relationships, the method will be optimised using a set of scans from multiple sclerosis brains before moving on to investigate a smaller set of ASD and control brains.

Introduction

Magnetic resonance imaging (MRI) is increasingly used to visualize the detailed structure of the cerebral cortex (e.g. cortical layers (Barazany and Assaf, 2011; Fatterpekar et al., 2002)). In particular, recent diffusion tensor imaging (DTI) studies have begun to investigate the diffusion signal in cortex (Anwander et al., 2010; Cohen-Adad et al., 2012; Hasan et al., 2007; Heidemann et al., 2009; Jeon et al., 2012; Jespersen et al., 2012; Kang et al., 2012; Kleinnijenhuis et al., 2012; Leuze et al., 2011; Leuze et al., 2012; McNab et al., 2013; Vrenken et al., 2006). This is of interest as diffusion metrics may be able to act as an in-vivo correlate of cortical microstructure, allowing more accurate differentiation of cortical regions and detection of subtle microstructural changes in development and pathology. Although diffusion in cortex exhibits some degree of fractional anisotropy (FA) (Anwander et al., 2010; Heidemann et al., 2009), mean diffusivity (MD) has initially been found to be more promising for this purpose as changes with age and pathology and differences across brain regions have been detected (Jeon et al., 2012). Several recent studies have shown that diffusion in the cortex is largely radial (i.e., perpendicular to the cortical surface) (McNab et al., 2009) while also showing regional variation (Anwander et al., 2010; Kang et al., 2012; Kleinnijenhuis et al., 2012; McNab et al., 2013) and this has been demonstrated to reflect the cortex's anisotropic cytoarchitecture (Jespersen et al., 2012; Leuze et al., 2012). Diffusion anisotropy in cortex has been characterised through measures monotonically related to the angle between the principal diffusion direction (PDD) and the cortical surface normal

(with smaller angles indicating a more tangential PDD). Such investigations have revealed areas of consistently radial diffusion in motor (Anwander et al., 2010; McNab et al., 2013) and prefrontal (Anwander et al., 2010) cortex, and mixed reports of either tangential (McNab et al., 2013) or radial (Kang et al., 2012) diffusion in other areas e.g. Heschl's gyrus.

It has been suggested that this directional diffusion may arise from the organisation and orientation of components of the cortical structure including axons, dendrites and the neurons themselves (Jespersen et al., 2012; Kang et al., 2012; McNab et al., 2013). The diffusion signal within the cortex shows regional variation (Anwander et al., 2010; Jeon et al., 2012; Kang et al., 2012; McNab et al., 2013) suggesting that by investigation of the cyto- and myelo-architecture, which also show regional variation (DeFelipe, 2005; Von Economo and Koskinas, 1925), we may begin to be able to shed light on the relationship between cortical architecture and diffusion in the cortex.

Previous work has demonstrated almost identical spacing between the axon bundles and the minicolumns (Casanova et al., 2008) supporting the idea that both are measuring different aspects of the same structure and suggesting that the regional and pathology-related variation observed in the cell body measurements should also be observable in measurements of the axon bundles. Both components were assessed in the present study. In addition, the effect on MRI diffusion measures of the width of the axon bundles themselves was also examined. As FA and MD have been shown to be sensitive to levels of myelin, at least in WM (Schmierer et al., 2008), myelin levels in the cortex were measured in the present study.

No studies have yet reported on the quantitative relationships between radial diffusivity in the cortex and minicolumn spacing, axon bundle width or spacing. Although diffusion

does not occur on a scale large enough to constitute a direct measure of these histological structures, variation in these structures will affect the proportion of water in different local environments (e.g. intra-axonal and extra-cellular) and so diffusion likely serves as a proxy for variation in tissue properties at the appropriate scale (Mori and Zhang (2006) estimate that water molecules move 10 μ m during a typical MR measurement time). For example, variations in axonal bundle width may reflect differences in numbers or widths of individual axons, either of which may affect the amount of myelination and/or membranes present (Peters et al., 2001; Zikopoulos and Barbas, 2010). The full range of factors influencing diffusion in the cortex is not fully understood and other factors such as the packing density of axons and relative volume fractions of these components may also contribute. This study set out to investigate the hypothesis that variation in the PDD relates to aspects of the minicolumn and axonal bundle organisation.

As a preliminary validation of the technique, post-mortem DTI scans from nine multiple sclerosis (MS) brains were used. Multiple sclerosis brains were used as the demyelination occurring in MS is suggested to create a greater range of myelin levels and possibly, values of cortical diffusion, than would be seen in controls, and so may make relationships easier to detect. The post-mortem MS scans had been obtained as part of a previous study which investigated the relationship between histology measures and white matter diffusion properties (Kolasinski et al., 2012). This applied PM diffusion imaging to white matter tracts in multiple sclerosis, based on the established sensitivity of MD and FA to demyelination and other aspects of white matter degeneration (Beaulieu, 2002; Schmierer et al., 2008). Measurements in the white matter tracts between cortical and subcortical grey matter areas correlated with standard histological measures in those areas (cortical thickness and cell density) (Kolasinski et al., 2012).

The present study investigated the correspondence between the histological and imaging measures in the two cortical regions investigated previously (Kolasinski et al., 2012): dorsolateral prefrontal cortex (dlPFC) and primary visual cortex (V1). Measures in these cortical regions were found to correlate with white matter myelination, providing a link between the cortex and varying levels of myelination. As MS is a demyelinating disorder, these areas were also chosen as areas of potential relevance in MS. In addition, these areas are reasonably well characterised and are known to represent a range of cortical cytoarchitectural arrangements (i.e. wider minicolumns in dlPFC and narrower minicolumns in V1). The present study additionally includes another well characterised comparison region - the primary auditory cortex within Heschl's gyrus (BA41) – which is of interest due to inconsistencies in previous reports on its PDD (Kang et al., 2012; McNab et al., 2013). Furthermore, inclusion of BA41 allows for greater continuity with the areas we are interested in investigating in ASD. Investigation of multiple cortical regions also allows us to explore the sensitivity of measures of diffusion to regional differentiation.

Methods

Patients/samples

Fixed whole brains from nine MS patients (Table 4.1) were obtained from the UK MS Tissue Bank (Imperial College, Hammersmith Hospital Campus, London). Brains were stored in 10% formalin before being transferred to a perfluorocarbon solution (Fomblin®

| Subject | Sex | Age | Hemisphere | Disease progression | Disease duration (yrs) | Time disease was progressive (yrs) ^a | Time in wheel-chair (yrs) ^a | PMI (hours) | SI (days) | Cause of death |
|---------|-----|-----|------------|---------------------|------------------------|-------------------------------------------------|----------------------------------------|-------------|-----------|--------------------|
| MS254 | F | 69 | R | Secondary | 37 | 12 | 7 | 66 | 1198 | MS |
| MS281 | F | 74 | L | Primary | 33 | * | 17 | 40 | 929 | Sepsis |
| MS314 | F | 78 | R | Secondary | 45 | 24 | 17 | 60 | 435 | Colonic carcinoma |
| MS316 | F | 79 | R | Secondary | 55 | 40 | 36 | 26 | 1052 | Pneumonia |
| MS322 | M | 72 | L | Secondary | 28 | 4 | * | 59 | 1201 | Pneumonia |
| MS332 | F | 50 | R | Secondary | 22 | 10 | 2 | 69 | 1134 | Breast cancer mets |
| MS334 | M | 66 | R | Secondary | 15 | * | 1 | 37 | 1126 | Prostate cancer |
| MS396 | F | 86 | R | Primary | 54 | * | * | 54 | 578 | Lymphoma |
| MS400 | F | 60 | L | Secondary | 11 | * | 7 | 21 | 539 | MS |

Table 4.1. Subject demographics. PMI=post-mortem interval (time between death and fixation); SI=scan interval (time between fixation and scanning). * indicates the data was not available

LC08; Solvay Inc.; Bollate, Italy) for scanning, which contributes no MRI signal and provides susceptibility matching to tissue (reducing image artifacts).

MRI scanning

Scanning was carried out by FMRIB scanning technicians on a Siemens Trio 3T scanner using a 12-channel head coil. Each scan session lasted approximately 24 hours. Diffusion weighted data were acquired using a modified spin-echo sequence with 3D segmented EPI ($T_E/T_R=122/530\text{ms}$, bandwidth=789 Hz/pixel, matrix size: 168x192x120, resolution 0.94x0.94x0.94mm). Diffusion weighting was isotropically distributed along 54 directions ($b=4500\text{s/mm}^2$) with six $b=0$ images. This protocol takes approximately six hours, and was repeated three times for 18 hours total diffusion imaging. Structural scans were acquired using a 3D balanced steady state free precession (BSSFP) sequence ($T_E/T_R=3.7/7.4\text{ms}$, bandwidth=302Hz/pixel, matrix size: 352x330x416, resolution 0.5x0.5x0.5mm). Images were acquired with and without RF phase alternation to avoid banding artifacts. This was averaged over eight repeats to increase signal to noise ratio. More details can be found in Miller et al. (2011).

Data was processed using the FMRIB software library (FSL) (Smith et al., 2004; Woolrich et al., 2009). The FSL diffusion toolbox was used to process diffusion weighted data, which incorporates an in-house processing pipeline to compensate for gradient-induced-heating drift and eddy-current distortions, to produce maps of fractional anisotropy (FA), mean diffusivity (MD) and the diffusion tensor components (Miller et al., 2011).

Cortical Diffusivity Analysis

Cortical diffusivity analysis consisted of three stages: masking of the region of interest (ROI), calculation of the diffusion metrics within the ROI using CHIPS (Cortical Hi-resolution Intensity Profile Segmentation; an in-house component of FSL) and extraction of the values to be compared with the histology measurements. CHIPS was developed specifically for this purpose and as the first project to use CHIPS the current project also involved some optimization of its application.

Masking the ROI

Cortical ROIs corresponding to those sampled histologically were delineated using manually created masks on the structural post-mortem images. By careful reference to photographic images of the physically cut coronal brain slice before and after the tissue block was removed, and the corresponding Nissl stained slide, the closest matching coronal slice of the structural MRI scan was identified. This was designated the central slice of the mask. The cortical diffusion analysis relies on calculating average values along cortical profiles running radially across the cortex within the area defined by the mask. This therefore requires a 3D mask to be created, as the cortical profiles cannot be assumed to lie entirely with a particular coronal slice of the structural MRI scan. In order to determine the optimal number of coronal slices to mask around this central slice the effect of increasing the number of slices masked was examined. A series of masks were created, covering 9, 11, 13, 15 or 17 slices, all centered around the same coronal slice. The mask covering 9 coronal slices was created first and subsequent masks were created by adding two coronal slices to the previous mask. CHIPS was then run to obtain the output measures of cortical diffusion. A number of voxels were identified in this central coronal slice (at least 35 voxels), and the value of this voxel compared across the outputs

from the different size masks. As can be seen in Figure 4.1 the mean difference between the value of a voxel and its value in the previous mask, decreased with increasing numbers of slices masked across diffusion metrics. Taking an average of the percentage change across the different measures of cortical diffusion, for each mask size separately revealed the smallest change was seen at 15 slices (0.277%; 9 slices: 5.022%; 11 slices: 0.635%; 13 slices: 0.468%; 17 slices: 0.409%). Therefore 15 slices was chosen as the optimal point by which the smallest changes were seen across the different diffusion metrics.

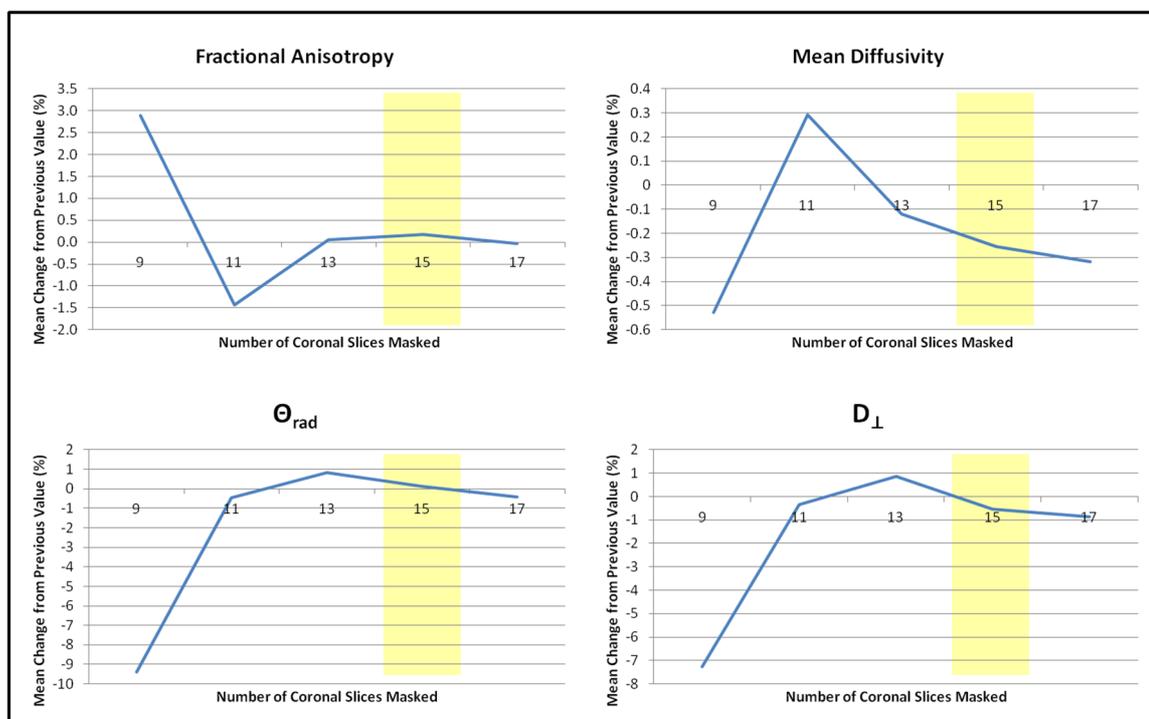


Figure 4.1. The relationship between the number of coronal slices included in the mask and the average change from the value in the previous mask for all four of the diffusion metrics being investigated. 15 slices was chosen as the optimal number of slices to be masked (highlighted in yellow).

Cortical ROIs were masked over 15 coronal slices of the MRI image centered around this slice, taking care to include only grey matter voxels to avoid contamination from white matter or CSF. The limits of the cortical ROIs were determined by careful comparison with the photographic images, and corresponding Nissl stained slides, in order to ensure the masked area matched the histologically sampled area.

Calculation of Diffusion Metrics

CHIPS was used to generate cortical profiles, i.e. lines across the cortex in a radial direction, perpendicular to the cortical surface (using a Laplacian potential model, based on (Jones et al., 2000)). Values for the diffusion tensor derived metrics were averaged along the cortical profiles, across the entire masked ROI, excluding regions near the ends of the ROI. The average value for each cortical profile was stored at the mid-point of the profile, creating a set of values arranged as a curve running parallel to the cortical surface (Figure 4.4). The metrics calculated were FA, MD and several measures relating to the principal diffusion component: namely the angle between the radial direction across the cortex and the principal diffusion direction (Θ_{rad}) (Figure 4.2); and the component of the principal diffusion (associated with the principal diffusion direction) that is perpendicular to this radial direction (D_{\perp}) (Figure 4.2). The latter gives a measure of how much of the diffusion occurring along the PDD is perpendicular to the radial direction across the cortex. These two measures were chosen as it is known that cortical regions largely differ in their cyto- and myelo-architecture in terms of horizontal spacing between these radial components, rather than the spacing of components in the radial direction itself. Therefore, it is expected that diffusion will be more or less restricted in this horizontal direction (i.e. tangential to the radial direction across the cortex) as a result of variations in the organisation and spacing of the radial barriers to diffusion provided by the cortical cytoarchitecture.

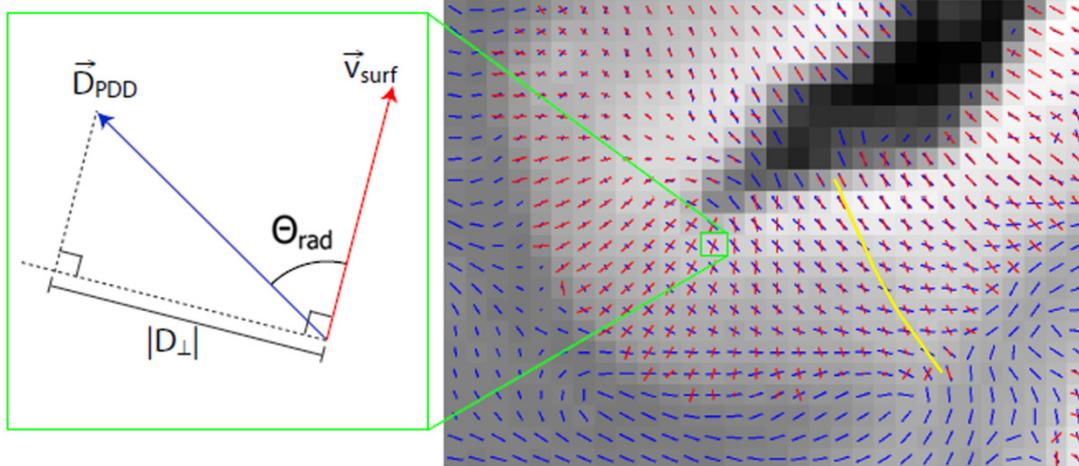


Figure 4.2. Example of the cortical diffusion data for one representative region (right), including an illustrative voxel example of the derived diffusion-based measures (left). Blue lines indicate the principal diffusion vector in each voxel: on the right, only the direction is indicated, while on the left the diffusion tensor component along the PDD vector (D_{PDD}) is shown. Red lines indicate the estimated radial direction perpendicular to the cortical surface (labelled v_{surf} on the left). The angle of radially, Θ_{rad} , in a voxel is the angle between the red and blue lines. The perpendicular diffusivity, D_{\perp} , is calculated by projecting D_{PDD} onto the plane perpendicular to v_{surf} . Both quantities are averaged along the radial direction across the cortex, as indicated for one set of voxels by the yellow line.

Extraction of values

For each location photographed for the minicolumn analysis, the corresponding area within the ROI mask (on the MRI scan) was visually identified through careful comparison with the photograph locations marked on the corresponding Nissl-stained slide. In order to guard against slight mismatches between the area photographed and the best-match voxel chosen within the MRI scan, it was decided to calculate an average over several voxels. A preliminary investigation was conducted in order to determine the optimal number of voxels to include in this average.

Five different averages were investigated as detailed in Table 4.2 below. Each consisted of a number of voxels in the slice that most closely matched the area in the slide (slice 0), with some averages also containing values from voxels in the slice anterior (slice -1) and posterior (slice +1) to slice 0.

| Average ID | Number of Voxels from: | | |
|------------|------------------------|---------|----------|
| | Slice -1 | Slice 0 | Slice +1 |
| 1 | 0 | 3 | 0 |
| 2 | 1 | 1 | 1 |
| 3 | 3 | 3 | 3 |
| 4 | 0 | 5 | 0 |
| 5 | 5 | 5 | 5 |

Table 4.2. Voxels used in calculating the averages illustrated in Figure 4.3 below.

For simplicity, this preliminary analysis was restricted to MD and FA values. A repeated measures ANOVA was conducted with the different averages as the within-subjects factor, for PFC and V1 separately for each of FA and MD, as PFC and V1 are expected to show different values. No difference was observed between the different averages (all $p > 0.1$). This result was also confirmed by looking at the data graphically (Fig 4.3). In order to balance against potential mismatches between location photographed and best-match voxel chosen both within a given coronal slice, and between coronal slices, average 3 was chosen as this averages both within and between coronal slices. In addition, this should give a reliable value representative of the location sampled histologically, while still remaining within 1mm of the location identified as the best match to the photograph.

In order to calculate the diffusion metric values for this location, we identified the voxel in the coronal slice most closely corresponding to the photograph location, along with the neighbouring voxel on either side (as shown in Figure 4.4). This coronal slice was designated the central slice. We then repeated this for the coronal slice immediately anterior and immediately posterior to this central slice. This identified a total of nine voxels (three from each coronal slice), the values of which were averaged to give a single value for each diffusion metric for that location (Figure 4.4). The visual matching performed between the photographed locations on the slides and the corresponding location on the MRI scans was not always perfect and so this averaging was performed in

order to make the measurement more robust as well as to minimise the influence of noise in the DTI data itself. For the measures relating to the direction of diffusion this local averaging additionally smoothes the data, ensuring only directionality with some local coherence will emerge and so guarding against the possibility that the deflection of the principal diffusion direction from the radial direction is random.

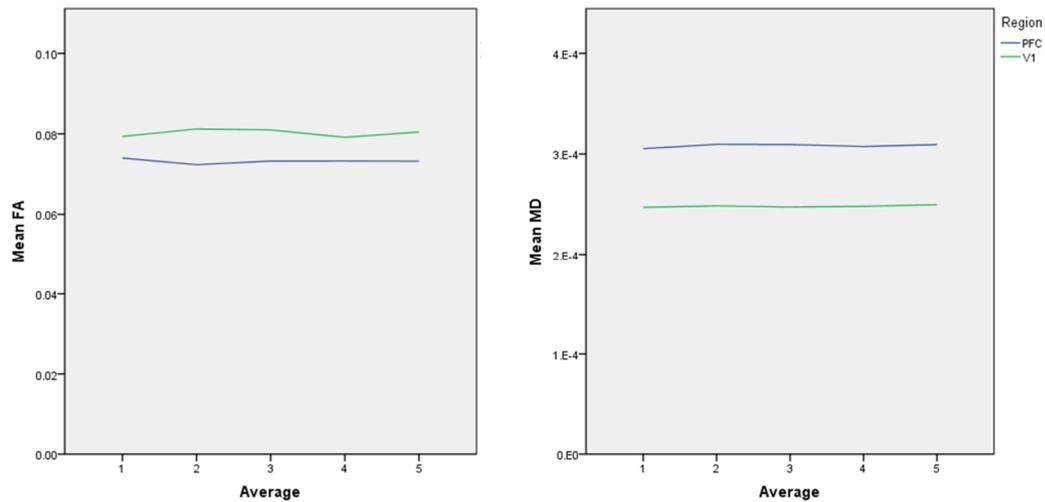


Figure 4.3. Mean FA and MD values generated by calculating an average over the voxels indicated in Table 4.2

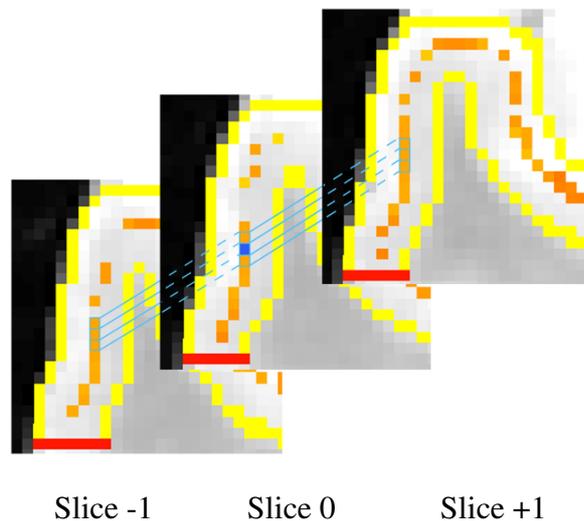


Figure 4.4. Example CHIPS output. The highlighted square (blue) denotes the closest match to the area photographed from the histological section. An average value for the location is calculated over this and the surrounding eight voxels from the mid-points of the cortical profiles (orange), as outlined in the figure.

A single value for each diffusion metric, for each ROI, was calculated by averaging the values obtained from the three photomicrograph locations sampled within the ROI. This not only adds further robustness against the small misalignments that may have occurred in matching the locations sampled histologically with the MRI voxels sampled for the diffusivity measures, but also allows for consistency with the histology measurements, which similarly calculate a single value for each ROI. Previous work has found that measures of the cyto- and myelo-architecture are relatively stable within a cortical subregion (e.g. Von Economo and Koskinas (1925)) indicating that it is valid to find an average value for that region.

Neurohistological sampling

Brains were sectioned coronally and the diagnosis of MS was confirmed by a clinical neuropathologist. Blocks of size 25mm x 25mm x 10mm were sampled for each of the three regions from one hemisphere per brain (a representative sample of hemispheres: 3 left, 6 right). Blocks and the surrounding tissue were photographed using an Olympus C-5050 digital camera for reference. dIPFC ROIs included the middle and superior frontal gyri bounded inferiorly at the paracingulate sulcus and inferior frontal sulcus. dIPFC blocks were sampled level with the cingulate gyrus (Figure 4.5). BA41 blocks incorporated Heschl's gyrus, bordered medially by the insula cortex and laterally by the planum temporale (Figure 4.5). V1 blocks were sampled along the calcarine fissure, level with the medium transverse occipital gyrus (Figure 4.5). ROI selection was confirmed cytoarchitecturally in accordance with Von Economo and Koskinas (1925).

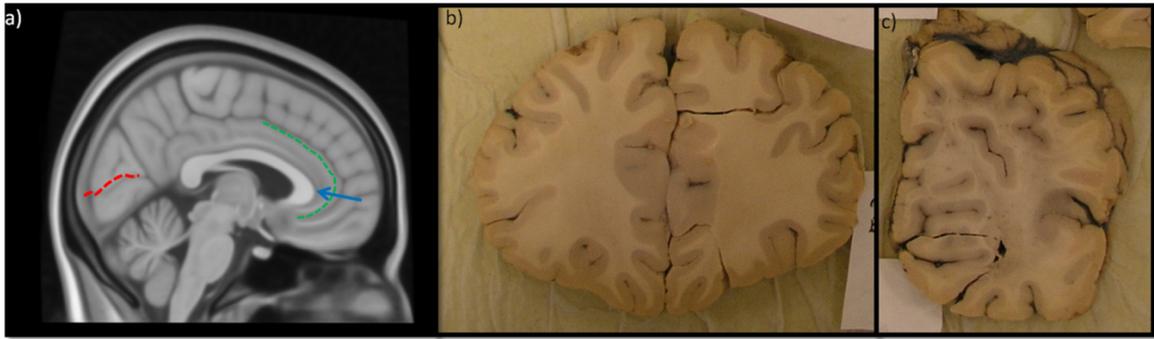


Figure 4.5. a) location of sulci used in delineating cortical regions of interest: red=calcarine fissure, green=paracingulate sulcus, the cingulate gyrus is indicated by the blue arrow. Example sections of b) dIPFC and c) V1

Tissue blocks were embedded in paraffin wax and serially sectioned at 10 μ m for the minicolumn analysis and quantification of myelin levels, and 30 μ m for the bundle measurements. Sections were stained with cresyl violet (CV; ThermoFisher Scientific, Waltham, MA, USA) for minicolumn analysis, anti-proteolipid protein stain (AbD AbSerotec, Oxford, UK) (anti-PLP) for light transmittance myelin quantification and Sudan black, a myelin sensitive lipophilic dye, for measurement of axonal bundles.

Minicolumn Analysis

Minicolumn width based on cell bodies was assessed using a semi-automated procedure as described in Chapter 3. Briefly this calculated a value for the minicolumn width consisting of the cell dense core region plus the associated neuropil space surrounding this. For each ROI, three photographs were taken, from a single slide where possible, and an average calculated over these to give a single value for each region.

Quantification of Myelin Levels

Cortical myelin content was assessed using light transmittance to quantify the intensity of myelin stain in anti-PLP stained sections. Data were collected using Axiovision v4.7.2 software on a PC receiving a signal from an AxioCam MRc (Carl-Zeiss, Jena, Germany) mounted on a BX40 microscope (Olympus, Japan) with a 10x objective lens. The set up

was calibrated in RGB mode with fixed white balance and incident light, using a standard slide/coverlip preparation and light filters (6%, 25% and 100% transmittance). For each ROI three measures of transmittance (T) were taken in each of layers III and V at locations matched to those photographed for the minicolumn analysis, using a 58, 240 μm^2 virtual frame on anti-PLP stained sections, and the resulting values averaged.

Axon Bundle Analysis

Axon bundle analysis was carried out as described in Chapter 3. Briefly, for each region three photographs were obtained through a 10x objective lens (resolution 1.10 μm , although the resolution achieved with real data will be lower due to the presence of noise) with an Olympus BX40 microscope, centred around layer V as the axon bundles were clearest there (Figure 4.6). Image locations were chosen to provide the best match to the locations photographed for the minicolumn analysis, and were obtained from a single slide where possible. Measurements of axonal bundle centre-to-centre spacing, and the width of the bundles themselves were made manually in Axiovision, using the in-built measurement tools (Figures 3.4, 3.5).

Pilot data revealed high reliability of this method, finding a high correlation ($r=0.737$, $p<0.001$) between measurements of photos taken on two different occasions. Coefficients of error were calculated according to the following formula, in order to assess the accuracy of axon bundle measurements. Coefficients of error were calculated for each cortical region using all the individual measurements made for that same region across all subjects (this resulted in a mean number of 49 values being used to calculate each coefficient of error for axon bundle spacing, and 31 values being used to calculate each coefficient of error for axon bundle width).

$$\text{Coefficient of error} = \frac{\text{Standard Error of the Mean}}{\text{Mean}}$$

A value of less than 0.1 is desirable. As can be seen in Table 4.3, values of less than 0.1 were obtained for both axon bundle width and spacing in all cortical regions, suggesting this method provides an accurate estimate of these measures. The values from the three photographs were then averaged to give a single value for bundle spacing and a single value for bundle width for each ROI.

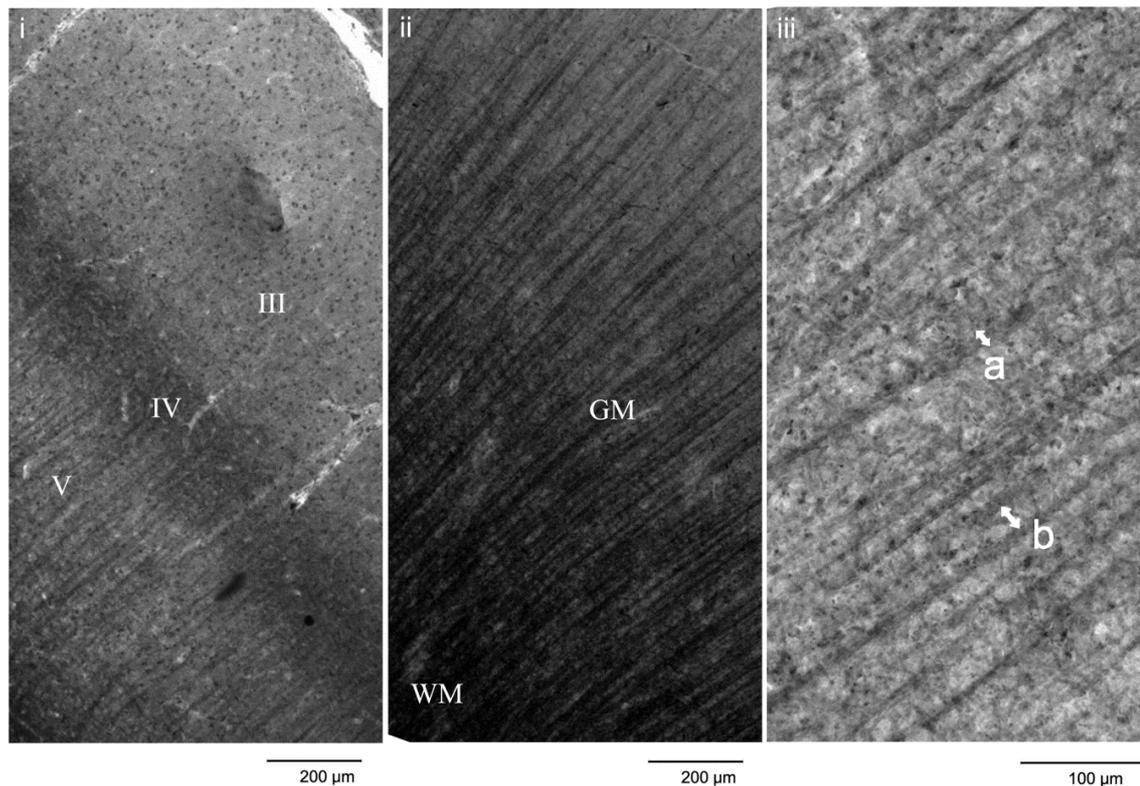


Figure 4.6. Sudan black stained section illustrating i) cortical layers, ii) tissue type and iii) measurements of axonal bundle width (a) and axonal bundle spacing (b), as indicated by the arrows.

For bundle width, this resulted in an average of $28 (\pm 5)$, $22(\pm 5)$, and $44 (\pm 5)$ bundles being sampled across the three photographs for dlPFC, BA41 and V1 respectively for each subject, constituting 94 bundles per case - comparable to the 100 bundles measured by Seldon (1981b). Coefficients of error were all below 0.1, which indicates an acceptable level of accuracy (Table II). For bundle spacing a mean of $42 (\pm 4)$, $39 (\pm 5)$ and $65 (\pm 8)$ measurements were made for dlPFC, BA41 and V1 respectively.

It was not possible to conduct a similar analysis to that performed by the CHIPS software to assess the orientation of the axon bundles within the cortex as CHIPS relies on calculating the geometrical vertical direction across the cortex from the GM/WM boundary to the pial surface from a three-dimensional dataset. Such a three-dimensional estimate is not possible in histological sections that have a limited depth (in this case 30 μ m), compounded by z-direction compression on the microscope slide, and the radial direction across the cortex is best determined from the radial microstructure itself. However, taking a subset of cases with a relatively uncurved section of cortex where it may be assumed that the three-dimensional geometric vertical is reasonably close to the two-dimensional estimate obtained from the histological section, we were able to measure the orientation of the axon bundles relative to this. This indicated that the axon bundles deviate from the radial direction across the cortex by an average of 3.50 (\pm 2.68) degrees.

Statistical Analysis

All data were analysed using SPSS v19 for Windows.

Relationship between histology and DTI - The relationship between the microanatomy and MRI diffusion measures across the full data set was investigated by correlation analysis using Pearson's Correlation Coefficient.

Mean regional differences - Regional differences in both histology and DTI measures were assessed using repeated measures ANOVAs, with cortical region as the within subjects factor, and significant main effects were followed up with post-hoc t-tests. As post-hoc tests to investigate the effect of cortical region involve three comparisons, no correction for multiple comparisons is required.

Histology measures – Relationships between histology measures were investigated using Pearson's Correlation Coefficient.

Results

Relationship between histology and DTI measures

None of the measures of cortical diffusion were found to be affected by post-mortem interval (PMI) although a significant correlation was observed between scan interval (SI) and FA ($r=-0.418$, $p=0.034$) only. Axonal bundle width showed a significant negative correlation with both Θ_{rad} ($r=-0.501$, $p=0.009$) and D_{\perp} ($r=-0.453$, $p=0.020$) (Figure 4.7). However, relationships between axonal bundle width and the two more commonly used DTI measures (FA and MD) were not significant.

For the spacing of the minicolumns, as assessed based on either cell bodies or myelinated bundles, the correlations did not reach significance with any measures of cortical diffusion. Levels of cortical myelination in layer V showed a relationship with FA ($r=0.471$, $p=0.020$), however this seemed to be due to one outlier and once this was removed the effect did not persist. No interactions between cortical diffusivity and myelination in layer III were observed.

The principle diffusion direction in the cortex has been shown to become more tangential proximal to the GM/WM boundary (Kang et al., 2012). In order to investigate the contribution of such fibre microarchitecture, the present study investigated the effect of excluding a band two voxels thick at the grey-white matter boundary. This resulted in a mean decrease in Θ_{rad} of 0.83 degrees (mean Θ_{rad} in PFC = 51.6 degrees with no erosion, 50.5 degrees with erosion of two voxels, mean Θ_{rad} in V1= 53.6 degrees with no erosion, 53.0 degrees with erosion of two voxels) (Figure 4.8).

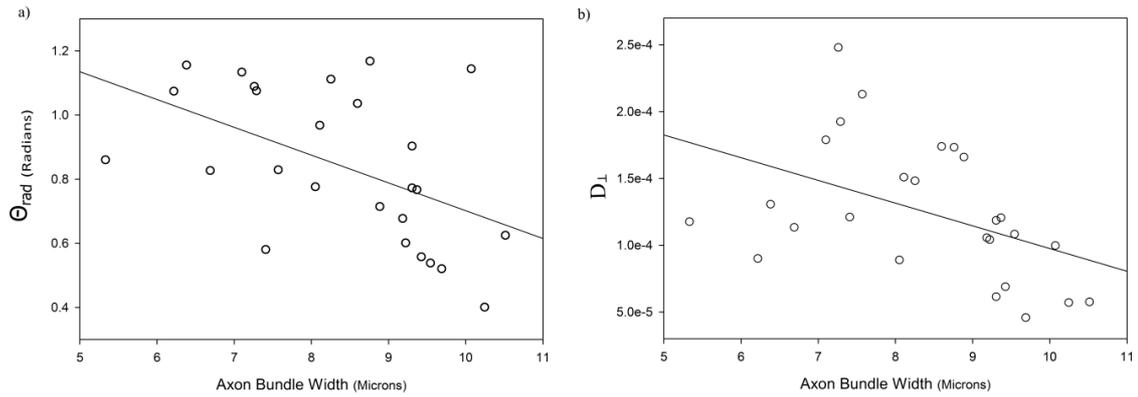


Figure 4.7. Least squares regression lines for the relationship between axon bundle width and a) Θ_{rad} , and b) D_{\perp} .

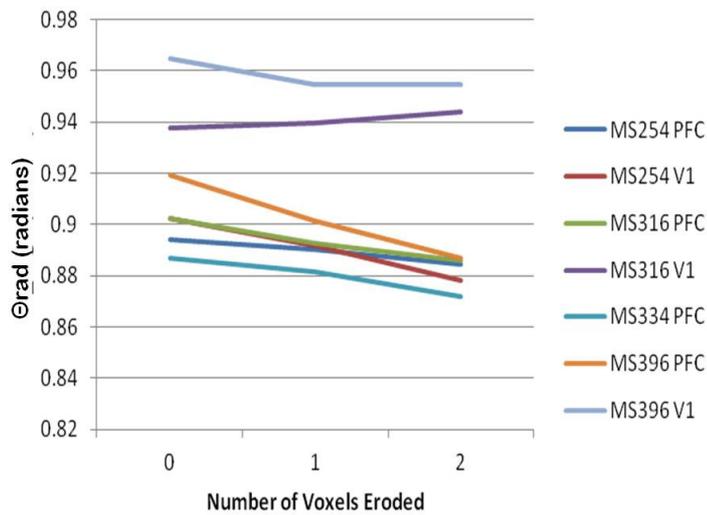


Figure 4.8. Effect on Θ_{rad} of eroding voxels at the grey-white matter boundary for a sample of regions.

One possible reason for the relationship between Θ_{rad} and bundle width is the presence of two components of cortical structure with different diffusion properties: isotropic diffusion in the space between the bundles and strongly anisotropic diffusion within the axon bundles. In the space between the bundles, the principal diffusion direction would be estimated to have random orientation. However, relative to any reference direction, a randomly chosen direction is far more likely to have a high angle than a low angle (e.g., there are more points at the equator of the Earth than at the poles). Thus, the angle of a purely random principal diffusion direction relative to the radial direction across the cortex is biased toward large angles. The proportions of the mixture of the isotropic

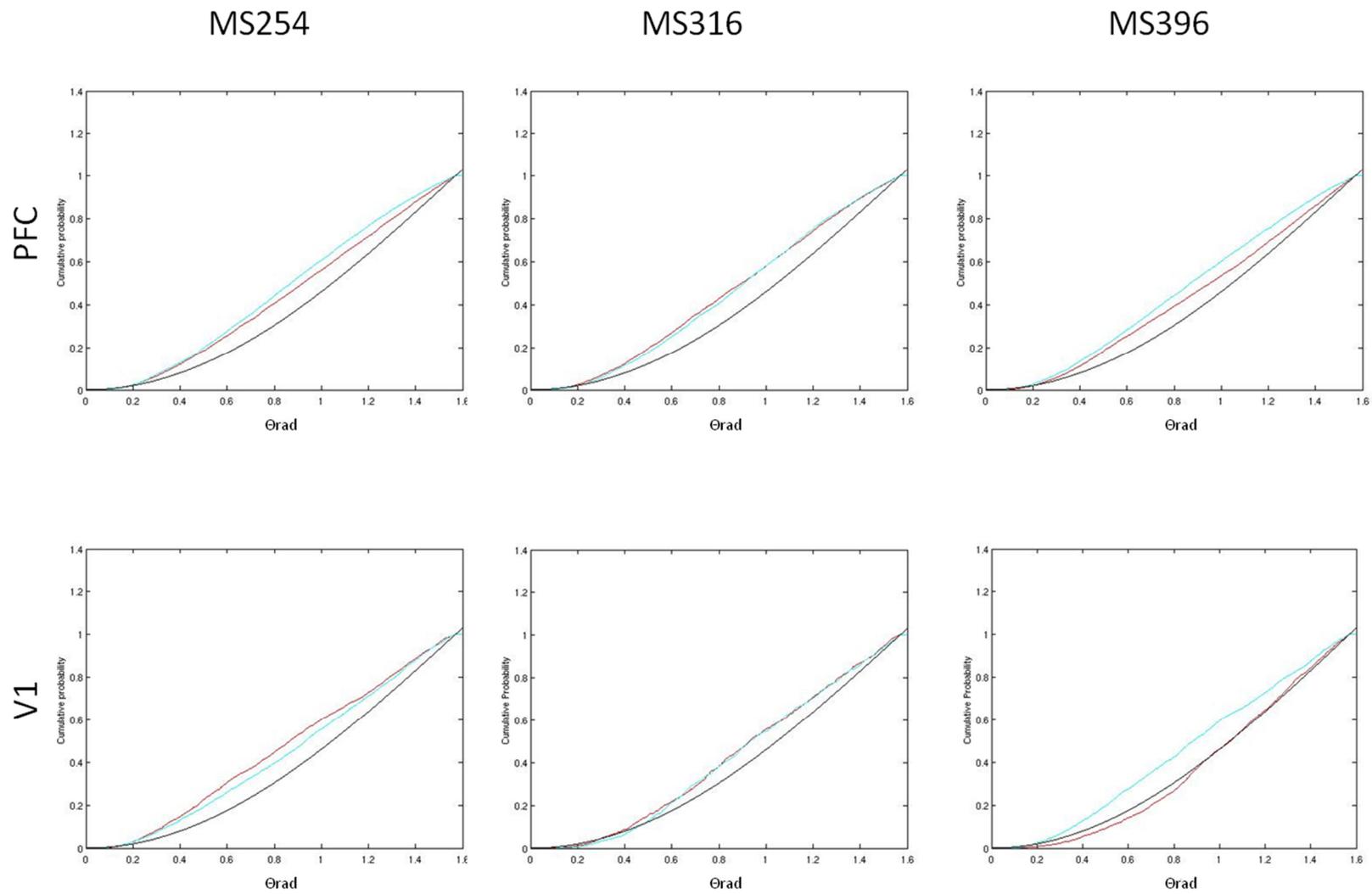


Figure 4.9. Distribution of Θ rad values for high FA voxels (shown in red) and low FA voxels (shown in cyan). $\sin(\Theta)$ rad is shown in black.

component (with its consequent tendency towards larger angles) and the anisotropic component (where the angle would be much lower) determines what the measured angle over the whole voxel will be. When the proportion of the anisotropic component increases (e.g. due to wider bundles or more closely spaced bundles) or the diffusion associated with the axon bundles becomes more anisotropic (e.g. due to tighter packing, more axons, or more myelin wrapping), Θ_{rad} will become smaller. In this scenario, the Θ_{rad} value is influenced by many factors, including the proportions of isotropic and anisotropic diffusion in the mixture. It follows that voxels that show strongly anisotropic diffusion (high FA values) would be expected to also show lower Θ_{rad} values (more radial diffusion). In order to explore whether this hypothesis could account for the observed results, we checked the distributions of Θ_{rad} values in high FA voxels and low FA voxels separately for a subset of cases. An essentially random PDD (as one would expect at low FA) would be characterized by a distribution of Θ_{rad} that is proportional to $\sin(\Theta_{\text{rad}})$. The voxels with high FA would be expected to show a higher proportion of lower Θ_{rad} values, with the distribution shifted to the left. The observed deviations were consistently shifted slightly towards the lower values, suggesting a systematic effect on the angle although the effect was subtle and there was little difference between high and low FA voxels (Fig 4.9).

Regional Differences

Repeated measures ANOVA revealed a significant main effect of region on all diffusion and histological measures (Tables 4.3, 4.4) (Figure 4.10). Primary visual cortex shows the narrowest minicolumns and narrowest axon bundles, with BA41 showing the widest spacing of axon bundles and the widest bundles. In terms of diffusivity measures, BA41 shows the largest FA, lowest MD, lowest D_{\perp} and the smallest Θ_{rad} value, suggesting a more radial principal diffusion direction.

| Region | Minicolumn Width (μm) | Axon Bundle Spacing (μm) | Axon Bundle Spacing CE | Axon Bundle Width (μm) | Axon Bundle Width CE | FA | MD | Θ_{rad} (rad) | D_{\perp} |
|--------|------------------------------------|---------------------------------------|------------------------|-------------------------------------|----------------------|------------------|--------------------|-----------------------------|--------------------|
| dIPFC | 37.7 (2.50) | 45.3 (3.74) | 0.025 | 8.2 (1.33) | 0.020 | 0.071 (0.019) | 0.00031 (0.000085) | 0.83 (0.22) | 0.00015 (0.000051) |
| BA41 | 33.7 (4.14) | 48.3 (6.82) | 0.025 | 9.6 (0.70) | 0.026 | 0.099 (0.023) | 0.00018 (0.000044) | 0.71 (0.24) | 0.00008 (0.000030) |
| V1 | 27.1 (3.38) | 28.6 (3.94) | 0.018 | 7.3 (0.84) | 0.013 | 0.091 (0.033) | 0.00024 (0.000066) | 0.97 (0.20) | 0.00014 (0.000038) |

Table 4.3. Measured variables within each region of cerebral cortex (mean and SD). Coefficients of error (CE) are provided for the histological measures of axon bundles. CE is not provided for automated image analysis measurements (minicolumn width and extracted DTI measures). Coefficients of error were calculated for each cortical region using all the individual measurements made for that same region across all subjects (photographs for axon bundle analysis were taken from a single slide where possible).

| | Histology Measures | | | Measures of Cortical Diffusion | | | |
|------------------|------------------------------|-----------------------------|-----------------------------|--------------------------------|-----------------------------|----------------------------|-----------------------------|
| | Minicolumn width | Bundle spacing | Bundle width | FA | MD | Θ_{rad} | D_{\perp} |
| Effect of region | F(2, 14)=22.523 P<0.001** | F(2,16)=45.076 P<0.001** | F(2,16)=18.345 P<0.001** | F(2,14)=11.663 P=0.009** | F(2,14)=21.513 P<0.001** | F(2,14)=4.809 P=0.026** | F(2,14)=10.136 P=0.002** |
| dIPFC vs. BA41 | T=2.189 P=0.065 | T= -1.125 P=0.293 | T= -3.586 P=0.007** | T= -16.098 P<0.001** | T=7.006 P<0.001** | T=1.125 P=0.297 | T=3.997 P=0.005** |
| V1 vs. BA41 | T= -4.299 P=0.004** | T= -7.340 P<0.001** | T= -6.559 P<0.001** | T= -0.923 P=0.387 | T=3.327 P=0.013* | T=2.810 P=0.026* | T=3.895 P=0.006** |
| dIPFC vs. V1 | T=9.013 P<0.001** | T=18.149 P<0.001** | T=2.228 P=0.056 | T= -2.807 P=0.021* | T=2.961 P=0.017* | T= -2.186 P=0.050* | T=0.951 P=0.388 |

Table 4.4. Overall effects determined by repeated measures ANOVAs are reported in the first row (effect of region). Post-hoc t-statistics are reported in the subsequent rows for specific region comparisons.

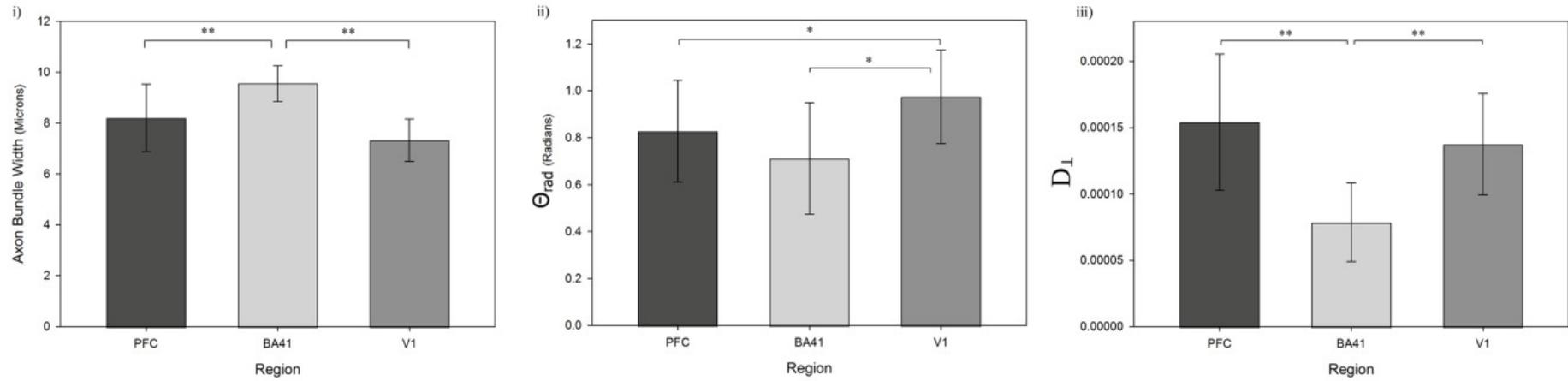


Figure 4.10. Regional differences, as shown by the post-hoc t-tests reported in Table 4.4, in i) axon bundle width, ii) Θ_{rad} and iii) D_{\perp} . * Denotes significance at the $p < 0.05$ level and ** denotes significance at the $p < 0.01$ level.

Relationship between histology measures

A strong positive correlation was observed between the width of the minicolumns in the cortex, as assessed by cell bodies, and the spacing of myelinated axon bundles ($r=0.745$, $p<0.001$) (Figure 4.11a). Bundle width also showed a positive correlation with bundle spacing ($r=0.556$, $p=0.003$) (Figure 4.11b) but the relationship between bundle width and minicolumn width assessed by cell bodies was not significant ($r=0.209$, $p=0.305$).

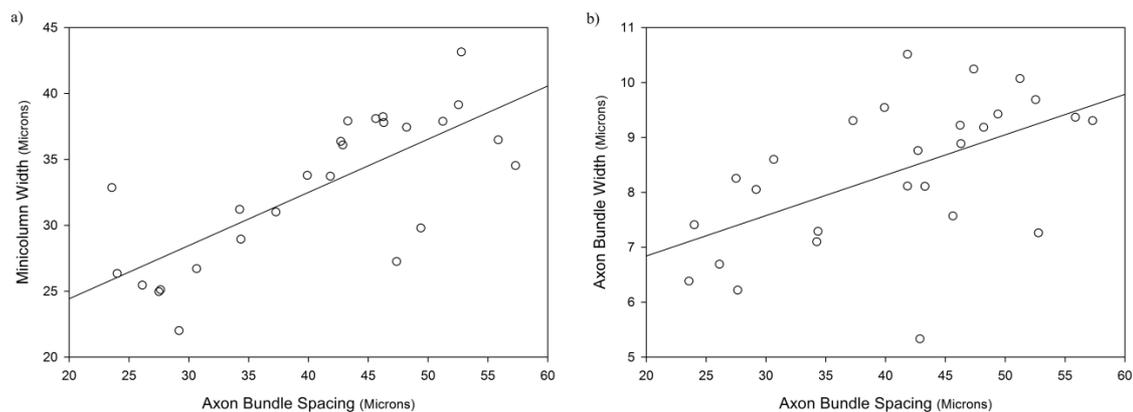


Figure 4.11. Least squares regression lines for the relationships between histological measures of cortical cytoarchitecture.

Discussion

The preliminary work conducted on these nine MS brains provides an initial investigation into the suitability of this method for investigation of cortical microstructure. Multiple sclerosis is neurological disorder with an estimated prevalence of 0.2% in the UK (Mackenzie et al., 2013). It is characterised by neurological and physical symptoms (including vision, balance and memory problems) followed by neurological deficits and increasing disability resulting from CNS demyelination (Rudick et al., 1997; Dymment et al., 2004). Although the focus in MS is often on white matter due to the demyelinating nature of the disorder, demyelination has also been demonstrated in

the grey matter (GM) using MRI, and histologically in areas appearing normal on MRI scans (normal appearing grey matter: NAGM) (Sbardella et al., 2013; Brink et al., 2004). Increases in MD and decreases in FA have been detected in the NAGM of MR patients, although the extent of the change seems to increase with increasing severity from clinically isolated syndrome (an initial onset of symptoms which may indicate the individual will go on to develop MS) through to relapsing-remitting MS (a form of MS where symptoms are experienced during relapses, periods of which are separated by remission where the symptoms are not apparent) and progressive MS (where rather than performance returning to baseline disability increases during the periods of remission) (Sbardella et al., 2013). As the patients included in this study were all diagnosed with either primary or secondary progressive MS (distinguished by whether the level of disability increases during remission from the outset: primary progressive, or following a period of relapsing-remitting MS: secondary progressive), it is likely that the measures of cortical diffusion we obtained are not representative of typically developing controls. Given this heterogeneity in forms of MS, and the possibility of moving between diagnoses (e.g. from relapsing-remitting to secondary progressive), it is well recognised that there is a great deal of variability in disease progression, meaning that measures of disease duration, or the time taken to reach different milestones (such as requiring a wheelchair) are not necessary good measures of clinical severity (Roxburgh et al., 2005). In order to investigate relationships between histological measures, measurements of cortical diffusion and disease severity a measurement of disability, such as that provided by the Expanded Disability Status Scale would be needed (Kurtzke, 1983).

Diffusion as an index of histology

The anatomical relationship with the diffusion signal was strongest for the measures relating to the axon bundles, likely due to the hindrance to water diffusion imposed by

the axonal membranes and myelin sheath. The lack of correlations between measures of cortical diffusion and overall levels of cortical myelination suggest that it is necessary to investigate more fine scale aspects of cortical architecture in order to elucidate the factors constraining diffusion in the cortex. It was not possible to determine in the present study whether increased bundle width reflected changes in the width of individual axons, number of axons in the bundle, packing density of the axons or thickness of myelin sheaths, and this is an area that has not been well documented in the literature. Cytoarchitectural evidence from monkey cortex suggests that with increasing axon diameter there is an increase in both myelin sheath thickness and number of lamellae surrounding the axon (Peters et al., 2001). A number of studies in a variety of animal species have found that G-ratio also increases with increasing axon diameter (Berthold et al., 1983; Chatzopoulou et al., 2008; Guy et al., 1989; Sunderland and Roche, 1958; Williams and Wendell-Smith, 1971) although one study found no difference (Friede and Samorajski, 1967). In humans this relationship has only been investigated in peripheral nerves, with one study finding an increase in G-ratio with increasing axon diameter (Friede and Beuche, 1985) and others finding a decrease in G-ratio (Buchthal and Rosenfalck, 1966; Jacobs and Love, 1985). Additionally, both demyelination and remyelination, as occurs in MS, would act to increase the G-ratio compared to the healthy state, as remyelinated axons are known to have thinner myelin sheaths than would be expected for the size of the axon (Liu et al., 2001). Therefore there are a number of processes that may be occurring at the level of the axon bundle, including changes in the width of individual axons, the number of coherently arranged membranes and myelin thickness. Although, it is unclear exactly how this relates to the net effect of axon bundles, minicolumns and other components at the scale of the MRI voxel, increases in axon size, myelin thickness and myelin layering in wider bundles may cause

tangential diffusion to be more restricted, leading to the lower D_{\perp} values observed in the present study.

As has been previously shown, the principal diffusion direction in the cortex becomes more tangential proximal to the GM/WM boundary which may be due to the sharp change in direction as fibres leave the white matter and enter the grey matter (Kang et al., 2012). In order to investigate the contribution of such fibre microarchitecture, the present study investigated the effect of excluding a band two voxels thick at the grey-white matter boundary. If this were due to a disproportionate effect of voxels containing fibres turning sharply from the white matter, the exclusion of voxels at the grey-white boundary would be expected to have a greater impact on Θ_{rad} values for area V1, where the cortex is thinner than in PFC. In fact area V1 was found to have a smaller average decrease than PFC (0.53 degrees in area V1 and 1.06 degrees in PFC) suggesting that this interpretation is unlikely to explain the relationship that was observed between bundle width and Θ_{rad} .

In contrast, although the data in Fig 4.9 only shows a subtle effect of FA on the distribution of Θ_{rad} , the direction of the effect is consistent with a model whereby isotropic diffusion is occurring in the space between the axon bundles, and anisotropic diffusion is occurring within the axon bundles themselves.

Regional Differences

Given the strong association between cortical cytoarchitecture and DTI measures it is not surprising that brain regions differ in their DTI characteristics as well as their cytoarchitecture. This is a finding of clinical relevance as selective regional changes may be informative in investigating differences associated with neurological disorders.

The present study found regional differences in minicolumn width between dlPFC and V1 that are similar to those that have been well characterized previously (Casanova et al.,

2008; Veluw et al., 2012). In this study, area 41 contained relatively wide minicolumns compared to dlPFC and V1. As our subjects were elderly, this may be explained by the age-related minicolumn narrowing normally observed in regions other than BA41 (Chance et al., 2006; Veluw et al., 2012). Axon bundle spacing showed a similar pattern, confirming the high correspondence between columnar width assessed by cell-body and axon-bundle-based measurements. The width of the axon bundles also differed between regions. This measure has generally been overlooked in the literature, so regional differences are not well characterized, although one study examining axonal bundles in BA41 found similar widths to those reported here (Seldon, 1981b).

The current findings of regional differences in the measures of cortical diffusion are consistent with a previous study which found the same pattern of MD across these regions i.e. MD was higher in frontal areas than occipital areas which in turn showed higher MD than temporal regions, in particular the superior temporal gyrus (Jeon et al., 2012). The present study also shows regional differences in Θ_{rad} - in particular, a significant difference between BA41 (most radial) and V1 (most tangential). For cortical FA, previous work has only looked at differences at the scale of, and between, cortical lobes and so is not comparable with the present work that looked at much more precisely defined regions. BA41 has been shown to have strongly radially organised cytoarchitecture (Sigalovsky et al., 2006; Von Economo and Koskinas, 1925), which may explain why it shows very directional diffusion (as suggested by high FA and low MD). Regional differences in the diffusion metrics observed in the present study and previous work (Anwander et al., 2010; Jeon et al., 2012; Kang et al., 2012; McNab et al., 2013) could arise from differences in the relative thickness of cortical layers introducing different mixtures of tangential and radial diffusion.

Previous studies investigating diffusion in the cortex have looked at the dot product between the PDD and the vector normal to either the cortical surface or an intermediate ‘surface’ calculated within the cortex (Anwander et al., 2010; McNab et al., 2013). We have calculated an equivalent quantity, but averaged across the entire thickness of the cortex. We argue that this provides a more representative picture of the organisation of the cortex in a particular region compared to a value calculated at the cortical surface alone. However, for areas such as V1, where a strong effect of cortical depth on angle values has been shown (Kang et al., 2012; Leuze et al., 2011), this may account for discrepancies between the current findings and those of previous work. Additionally, the present study uses a much smaller voxel size than previous work, which may also contribute to the greater range of values found here.

Previous findings in BA41 have been inconsistent, with McNab et al (2013) highlighting Heschl’s gyrus as displaying a notably tangential PDD. In contrast, Kang et al (2012) note that the poleward side of Heschl’s gyrus displays particularly radial values. Although we sampled across Heschl’s gyrus, due to the constraints on suitable regions for histological analysis and the wish to avoid straying into the association cortex of the planum temporale, it was necessary to sample more often from the poleward side. This may explain why there is a degree of radial diffusivity on average. Furthermore, given the finding of an effect of cortical depth on PDD (Kang et al., 2012) it is possible that these conflicting findings are due to variation in the position of sampling within the cortical depth, whereas our average across the entire cortical depth may give a good picture of the average structural orientation for a given region. Indeed, our findings are consistent with the detailed anatomical investigation of BA41 conducted by Seldon (1981b) which found the majority of axons were oriented radially. Similarly, the reference work of von Economo and Koskinas (1925) illustrated Heschl’s gyrus as

having well defined, radially-oriented myelinated bundles which would be expected to lead to a radial PDD, as found in the present study.

Implications for DTI

The findings of the present work suggest great potential for this method to allow the investigation of the microstructure of the cortex using DTI. As has been demonstrated here, cortical diffusion is particularly sensitive to axon bundle width, something that could be altered in disorders such as MS where demyelination is occurring, although future studies would need to investigate that further. It is hoped that these initial findings will provide a basis for future work to continue to investigate how measures of diffusion relate to the underlying microstructure, something that is not fully understood in white matter and virtually unstudied quantitatively in cortical grey matter and whether these measures may be able to act as potential biomarkers for particular disorders.

The finding of regional differences in measures of cortical diffusion opens up the possibility of incorporating such measures into algorithms for cortical parcellation. Currently these depend on sulcal and gyral landmarks, which do not always correspond exactly to cytoarchitectural distinctions between areas (Roland et al., 1997). Therefore, inclusion of measures of cortical diffusion, which show regional differentiation, may help to refine cortical parcellations based on these gross sulcal and cortical landmarks.

Relationships between histology measures

The present finding of a correlation between spacing of the minicolumns based on cell bodies and axon bundles is consistent with what is known about the structure of the minicolumn (Buxhoeveden and Casanova, 2002; Mountcastle, 1997) and previous work comparing the two measurements (Casanova et al., 2008).

In the present study the width of bundles increased with spacing between the bundles, but it was not possible to determine whether this reflected a greater number of individual fibers within the bundle or less dense packing of the same number of fibers. Understanding this may shed light on the functional implications of such regional variation. It has been suggested that narrowly spaced minicolumns have more overlapping activations, with functional implications for information processing (Chance et al., 2012; Harasty et al., 2003) and so minicolumns functioning less independently may have fewer axons in their bundles due to the greater redundancy in their information output. This would be of particular relevance to disorders such as autism where one of the most prominent neuroanatomical hypotheses is concerned with altered minicolumn organisation (Casanova et al., 2002b) and connectivity in fiber tracts (axon bundles) (Tommerdahl et al., 2008).

Limitations

The current study has been limited to investigating three regions of the cortex (dlPFC, V1, and BA41) due to the time-consuming nature of the manual steps involved in both histological processing and image analysis. Future studies across the whole brain would depend on development of robust, automated image analysis tools for segmentation and registration that can be applied to post-mortem data, as well as advances in histological processing hardware.

Although data of this kind is essential in elucidating the link between histology and features detectable in DTI, post-mortem tissue is known to show altered diffusion properties (Miller et al., 2011). Studies examining these changes in WM have demonstrated reductions in both FA and MD, e.g. (Miller et al., 2011; Schmierer et al., 2008), but this has been much less studied in GM. McNab et al. (2013) demonstrated a

high degree of radially in primary motor cortex for both in-vivo and post-mortem tissue. The finding of tangential diffusion in somatosensory cortex is not so uniformly replicated in the post-mortem tissue but this may be due to the increased chance of erroneous surface placement, and consequent calculations of the radial directions, when using surface based analysis on ex-vivo data (McNab et al., 2013) – a problem circumvented here by manual definition of cortical ROIs. Previous work has investigated the effect on diffusivity measures in post-mortem tissue of parameters such as post-mortem interval (PMI; time between death and fixation) and scan interval (SI; time between death and scan), with some studies, e.g. (D’Arceuil and de Crespigny, 2007; Miller et al., 2011), finding a dependence of diffusivity measures on PMI although other studies have suggested that these remain relatively constant after fixation, e.g. (Kim et al., 2009). It is worth noting that tissue volumetric change due to fixation (i.e. shrinkage) stabilizes within a few weeks (Quester and Schröder, 1997) and all of the cases in this study had been fixed for little more than the three years that Dyrby et al (2011) suggest is the length of the initial period of stable SI. Nonetheless, the present study tested for correlations between diffusivity measures and both PMI and SI, finding that only FA showed a relationship with SI. Although our previous investigation of this dataset showed effects of PMI in the WM, it is not surprising that the effects of PMI on diffusivity measures in the cortex are different. The brains used in this study were immersion fixed which causes the GM to come into contact with the fixative immediately whereas it has to penetrate through to the WM, effectively resulting in a more extended PMI. Overall there are reasonable grounds to expect a fair degree of correspondence between cortical diffusivity measures obtained in-vivo and those obtained from post-mortem tissue (including that with varying PMI and SI), although care should still be taken in relating our findings in the cortex to in-vivo data (particularly when considering FA) and future research should

focus on clarifying how diffusivity measures in the cortex differ between in-vivo and ex-vivo data.

One final limitation of this study was that in calculating D_{\perp} we were only able to measure the component of the diffusion along the PDD that can be projected onto the perpendicular direction, rather than being able to take diffusion that is orthogonal to the PDD into account. However, this is consistent with the calculation of Θ_{rad} in this study and similar measures of ‘radiality’ in other studies (Kang et al., 2012; McNab et al., 2013), which use only the PDD.

Conclusions

We describe a novel approach to the analysis of high-resolution MRI diffusion data in the cortex that is sensitive to myeloarchitecture in the human brain using DTI, with histologically-measured widths of axonal bundles associated with the principal direction of diffusion in the cortex. Further, we demonstrate regional differences in these measures of cortical diffusion. The current work used MRI of post-mortem tissue to enable the histological comparisons, but future application of this approach to sufficiently high-resolution in-vivo MRI scans would open up the possibility of detecting changes occurring in both normal and pathological development. The correlations with axonal bundles indicate that this technique may be of particular interest in demyelinating or connective disorders, such as MS and autism.

Chapter 5:

Post-mortem Scanning - Autism

Introduction

The investigation of measures of cortical diffusion in a pilot data set of MS scans in the previous chapter (Chapter 4) indicates that cortical diffusion is sensitive to the myeloarchitecture of the human brain, as well as differences between cortical regions.

Given the previous findings of minicolumnar differences in ASD (Buxhoeveden et al., 2006; Casanova et al., 2006a; Casanova et al., 2002b, c; Casanova et al., 2010; Casanova et al., 2006b) and suggestions of altered myelination (Zikopoulos and Barbas, 2010), along with the findings of altered axonal bundle organization (Chapter 3) analysis of cortical diffusion could prove a promising method for characterising microstructural differences between a set of ASD and control brains. In addition, measures of cortical diffusion may be useful in investigating previous claims of attenuated, or absent, cortical differentiation (Voineagu et al., 2011; Ziats and Rennert, 2013).

Six regions will be investigated in the present study. BA11, BA40, BA41 and PT will be included for a number of reasons. Firstly these are regions involved in processes known to be altered in ASD; BA11 and BA40 are involved in social processing, PT is involved in language, and BA41 is primary auditory cortex and is included due to auditory abnormalities that have been reported in ASD (Klintwall et al., 2011). They also represent different types of cortex; BA41 is a primary sensory region, PT is unimodal association cortex and both BA11 and BA40 are heteromodal association cortex, and minicolumnar and axonal bundle organisation has been investigated in all four of these regions (Chapter 3). In addition, PFC and V1 will also be included since cortical

diffusion within these regions has previously been investigated in the pilot MS data set (Chapter 4).

As the prime objective of this study is to investigate the ASD and control cases, rather than to enable comparisons with the set of MS brains, methodological consistency with the ASD and control cases studied in Chapter 3 will be prioritised. However, the correlations observed between histology and DTI measures in the MS data will be used to inform investigation of data from the ASD and control cases.

The specific aims of this study are therefore:

- 1) To investigate the sensitivity of measures of cortical diffusion to diagnostic differences. If the correlations between axon bundle width and Θ_{rad} and D_{\perp} are also present in the ASD data then, given the finding of narrower axon bundles in ASD reported in Chapter 3, larger values of Θ_{rad} and D_{\perp} would be expected to be seen in ASD.
- 2) To investigate the sensitivity of measures of cortical diffusion to regional variation. Although regional variation in minicolumnar structure is present in ASD (Chapter 3), several gene expression studies have suggested that cortical regional variation is attenuated in ASD (Voineagu et al., 2011; Ziats and Rennert, 2013). As minicolumn width has been shown to follow a hierarchical pattern (columns are narrowest in primary sensory areas and widest in heteromodal association cortex) cortical regions will be grouped according to their cortical hierarchy (primary sensory, unimodal association and heteromodal association cortex). It is expected that regional differences will be observed in ASD, but that

the magnitude of the differences between primary sensory, unimodal association and heteromodal association cortex will be smaller than those seen in controls.

- 3) To investigate the relationship between DTI and histology measures in ASD and control cases. As the preliminary work on MS data reported above indicates correlations between axon bundle width and both Θ_{rad} and D_{\perp} , the relationship between these variables will be of particular interest in the ASD and control data set. The model proposed above suggests the diffusion signal seen at the level of the whole voxel is a product of more anisotropic diffusion associated with the axon bundles themselves, and more isotropic diffusion in the space between the axon bundles. It is therefore reasonable to assume that if the ratio of axon bundle width to the space between bundles changes then this will affect the relationship between measures of cortical diffusion and these histological parameters. The present study will therefore investigate whether there is a difference in the ratio of axon bundle width to spacing between bundles between different regions of cortex. If a difference is found then the relationships between histology and measures of cortical diffusion should be considered separately for each region.
- 4) To confirm the existence of a strong correlation between minicolumn width as assessed based on cell body and axon bundle based measurements. As this relationship is thought to arise from the intrinsic structure of the cortical minicolumns this relationship would be expected to be seen in both ASD and control groups.

Methods

Subjects

Fixed whole brains or whole hemispheres for six ASD subjects and six controls (Tables 5.1, 5.2) were obtained from the Oxford Brain Bank. Brains were stored in 10% formalin before being transferred to a perfluorocarbon solution (Fomblin® LC08; Solvay Inc.; Bollate, Italy) for scanning, which contributes no MRI signal and provides susceptibility matching to tissue (reducing image artifacts).

MRI Scanning

For 11 cases scanning and pre-processing of the diffusion weighted data was carried out using the same procedure as for the MS brains (Chapter 4). Due to a change in scanning protocols CTL05 was scanned using a steady state free procession protocol and so DTI data from this case was not included in the present analysis.

Cortical Diffusion Analysis

Cortical ROIs were masked using the same procedure as for the MS scans (Chapter 4), and diffusion metrics calculated using CHIPS. Values were again extracted from the nine voxels centered around the voxel best matching the location photographed for histological analysis (Figure 4.4).

| Subject | Sex | Age (years) | Hemisphere | Brain weight (g) | Post-mortem Interval (hours) | Scan Interval (days) | Cause of Death |
|---------|-----|-------------|------------|------------------|------------------------------|----------------------|-------------------------------|
| ASD01 | M | 19 | L | 1243 | 6 | 508 | * |
| ASD02 | M | 22 | R | 1606 | 48 | | * |
| ASD03 | F | 44 | R | 1592 | 48 | 548 | Acute hypoxic encephalopathy |
| ASD04 | F | 44 | L | 1412 | 48 | 95 | Diffuse large B cell lymphoma |
| ASD05 | M | 14 | L | * | 72 | 2757 | * |
| ASD06 | F | 60 | R | * | * | * | * |
| CTL01 | M | 73 | L | * | 24 | 48 | * |
| CTL02 | F | 88 | R | 1103 | 24 | 111 | * |
| CTL03 | M | 68 | R | 1500 | 48 | 111 | Choriocarcinoma |
| CTL04 | F | 82 | L | * | 48 | 95 | * |
| CTL05 | F | 68 | R | 1295 | 48 | 73 | Carcinoma of pancreas |
| CTL06 | F | 48 | * | 1143 | 48 | 81 | Anti-synthetase syndrome |

Table 5.1. Demographic information, * indicates data was not available.

| Case | ADI-A | ADI-B | ADI-C | ADI-D | Seizures |
|-------|-------|-------|-------|-------|----------|
| ASD01 | 26 | 12 | 2 | 5 | No |
| ASD02 | 10 | 11 | 5 | 2 | No |
| ASD03 | * | * | * | * | No |
| ASD04 | 26 | 10 | 3 | 3 | No |
| ASD05 | * | * | * | * | Yes |
| ASD06 | * | * | * | * | No |

Table 5.2. Clinical information for ASD cases. * indicates data was not available.

Neurohistological sampling

Brains were sectioned coronally and blocks of size 25mm x 25mm x 10mm were sampled for each of the five regions from one hemisphere per brain. Blocks and the surrounding tissue were photographed using an Olympus C-5050 digital camera for reference. dIPFC ROIs included the middle and superior frontal gyri bounded inferiorly at the paracingulate sulcus and inferior frontal sulcus (Figure 4.5). dIPFC blocks were sampled level with the cingulate gyrus. BA41 blocks incorporated Heschl's gyrus and the

planum temporale. BA41 is bordered medially by the insula cortex and laterally by the planum temporale. The anterior boundary is marked by the first transverse sulcus and the anterior boundary by Heschl's sulcus (Figure 2.3). The planum temporale is located on the lower bank of the Sylvian fissure and bounded posteriorly by its ascending ramus (Figure 2.3). The anterior boundary is formed by Heschl's gyrus. V1 blocks were sampled along the calcarine fissure, level with the medium transverse occipital gyrus (Figure 4.5). BA11 blocks were sampled from the gyrus rectus, bounded medially by the midline, posteriorly by BA25, separated laterally from BA10 by the olfactory sulcus and dorsally from BA12 by the superior orbital sulcus (Figure 2.3). BA40 was defined as the supramarginal gyrus, which is bounded superiorly by the intraparietal sulcus, inferiorly by the sylvian fissure, anteriorly by the postcentral sulcus and posteriorly by the Jensen sulcus (Figure 2.3). We were particularly interested in area Pfm of BA40, which is defined cytoarchitecturally but is located towards the posterior part of the supramarginal gyrus. Therefore, BA40 was sampled from the area posterior to end of the Sylvian Fissure. ROI selection was confirmed cytoarchitecturally in accordance with Von Economo and Koskinas (1925).

All tissue was sucrose protected (30% w/v) prior to freezing to minimise artefacts. 30µm sections were cut and stained with cresyl violet (CV; ThermoFisher Scientific, Waltham, MA, USA) for minicolumn analysis and Sudan black, a myelin sensitive lipophilic dye, for measurement of axonal bundles.

Histological Analysis

Measurements of minicolumn width and axonal bundle width and spacing were made as for the MS tissue (Chapter 4). Due to the finding of no relationship between measures of myelination and DTI measures, myelination was not assessed in the ASD and control

cases. Although no relationship was detected with the measures of minicolumn width based on assessment of cell bodies, this was still measured in the ASD and control cases. This was partly due to the strong relationship between bundle spacing and minicolumn width observed in the MS data, which suggests relationships may exist between the minicolumn width and DTI measures, but also as the measurements of minicolumn width had been collected from some of these areas as part of the larger ASD and control sample (Chapter 3).

The relationship between histology and DTI measures was not investigated across MS, ASD and control cases due to differences in histological preparation between samples. MS tissue had been paraffin embedded as part of the previous study (Kolasinski et al., 2012) whereas ASD and control tissue was frozen as this has been shown to reduce shrinkage in the z-direction compared with paraffin embedding (Gardella et al., 2003; Hatton and Von Bartheld, 1999).

In addition, while 10 μ m sections were cut from the paraffin embedded MS tissue for minicolumn analysis, 30 μ m sections were cut from the frozen ASD and control tissue. This was to ensure comparability with previous measurements of minicolumn width from our group, specifically with the measurements of minicolumn width being made from the larger sample of ASD and control tissue (Chapter 3). In order to assess the impact of section thickness on minicolumn measurements a preliminary investigation was conducted using paraffin embedded MS tissue sectioned at 10 μ m, 15 μ m, 25 μ m and 30 μ m. A repeated-measures ANOVA with section thickness as the within-subjects factor and cortical region (PFC or V1) as the between-subjects factor revealed a significant effect of section thickness ($p < 0.001$) as well as an interaction between cortical region and section thickness ($p = 0.001$). As can be seen in Figure 5.1 larger estimates of minicolumn width are found from thinner sections, possibly due to some minicolumns falling out of

the plane of sectioning. This suggests that the estimated of minicolumn width obtained from the 10 μ m MS sections may be an over-estimate of the width. Given that the curves for PFC and V1 (Figure 5.1) remain similarly separate across the different section thicknesses this suggests that all measurements are affected similarly, and so measurements at 10 μ m sections still provide a useful estimate of minicolumn width. In contrast, the differences generated by the different preparations cannot be quantified, and would introduce differences in measurements of minicolumn width even at the same section thickness. Therefore, making meaningful comparisons between the previous MS data and current ASD and control data would be impossible.

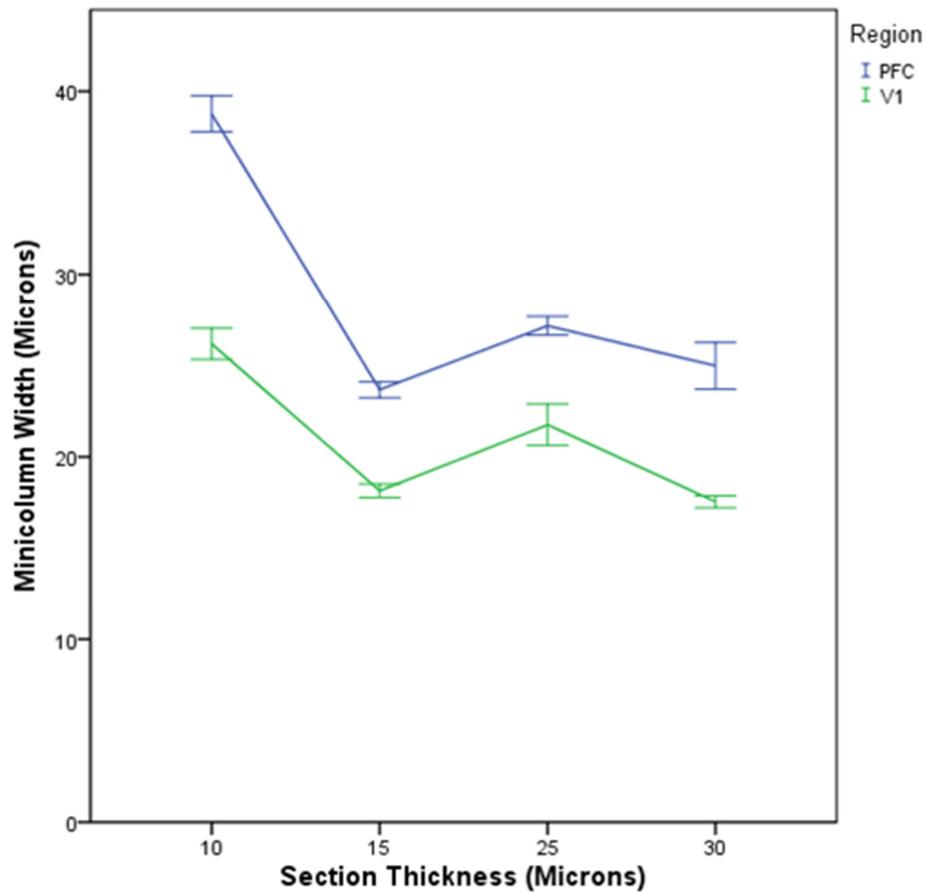


Figure 5.1. Relationship between minicolumn width and section thickness for both PFC and V1. Error bars represent the standard error of the mean.

Statistical Analysis

All data were analysed using SPSS v19 for Windows.

Diagnostic and regional differences – Repeated-measures ANOVA, using cortical region as the within-subjects measure and diagnosis as the between-subjects measure, was used to investigate the diagnostic and regional differences in both the measures of histology and cortical diffusivity. Effects of including age and brain weight as covariates were also explored.

Relationship between histology and DTI - The relationship between the microanatomy and MRI diffusion measures across the full data set was investigated by correlation analysis using Pearson's Correlation Coefficient.

Relationships between histology measures – Relationship between histology measures was investigated by correlation analysis using Pearson's Correlation Coefficient.

Relationship between DTI measures and clinical variable – Clinical variables (ADI-R scores) were only available for three of the ASD cases and so relationships with DTI measures were not investigated in this sample. Relationships between histology and clinical measures were not investigated in this subset of cases as they had already been explored in the larger set of cases (Chapter 3).

Results

Diagnostic and Regional Differences

Regional differences were investigated using repeated-measures ANOVAs with cortical region as the within-subjects factor and diagnosis as the between-subjects factor. Due to

scan artefacts or poor tissue quality measurements were only available for BA11 for 3 out of 6 control cases. Therefore BA11 was excluded from this analysis. A main effect of region was found for all histology measures (all $p < 0.015$) and for MD ($p = 0.033$) although not for the remaining DTI measures (all $p > 0.1$) (Table 5.3). Minicolumn width was the only measure to show a trend effect of diagnosis ($p = 0.058$) and a trend towards an interaction between cortical region and diagnosis ($p = 0.063$). Post-hoc investigation of the interaction between cortical region and diagnosis for minicolumn width revealed wider minicolumns in ASD in PFC ($t = 3.160$, uncorrected $p = 0.01$; Bonferroni corrected significance level for 5 tests = 0.025), BA41 ($t = 2.638$, uncorrected $p = 0.025$; Bonferroni corrected significance level for 5 tests = 0.025) and PT ($t = 2.218$, uncorrected $p = 0.051$; Bonferroni corrected significance level for 5 tests = 0.025). Post-hoc investigation of the difference between cortical regions revealed that this effect was primarily driven by different values in V1 for the histology measures and PFC for MD (Figure 5.2).

| | Histology Measures | | | Measures of Cortical Diffusion | | | |
|---------------------------------|-------------------------------|----------------------------|--------------------------|--------------------------------|------------------------------|-------------------------|-----------------------------|
| | Minicolumn Width | Bundle Spacing | Bundle Width | FA | MD | Θ_{rad} | D_{\perp} |
| Effect of region | F(2.0,18.2)=44.0 P<0.001** | F(4,40)=29.98 P<0.001** | F(4,40)=3.57 P=0.014* | F(4,32)=0.24 P=0.914 | F(2.0,15.7)=4.28 P=0.033* | F(4,32)=1.89 P=0.136 | F(2.4,19.2)=2.16 P=0.135 |
| Region by Diagnosis interaction | F(2.0,18.2)=3.23 P=0.063 | F(4,40)=0.50 P=0.734 | F(4,40)=0.94 P=0.453 | F(4,32)=1.05 P=0.396 | F(2.0,15.7)=1.36 P=0.285 | F(4,32)=1.91 P=0.133 | F(2.4,19.2)=1.34 P=0.289 |
| Effect of diagnosis | F(1,9) = 4.71 P=0.058 | F(1,10)=0.25 P=0.631 | F(1,10)=0.04 P=0.849 | F(1,8)=0.03 P=0.879 | F(1,8)=0.069 P=0.431 | F(1,8)=3.02 P=0.120 | F(1,8)=0.78 P=0.402 |

Table 5.3. Overall effects determined by repeated-measures ANOVAs are reported in the first row (effect of region). Post-hoc t-statistics are reported in the subsequent rows for specific region comparisons. * indicates significance at the p<0.05 level; ** indicates significance at the p<0.01 level.

| | | PFC | BA11 | BA40 | BA41 | PT | V1 |
|------------------------------------|-----|-----------------------|----------------------|----------------------|-----------------------|----------------------|----------------------|
| Minicolumn width (μm) | ASD | 38.9 (4.2) | 35.3 (3.9) | 35.2 (4.2) | 32.4 (3.5) | 35.4 (3.7) | 24.1 (2.3) |
| | CTL | 32.8 (2.3) | 31.1 (1.9) | 32.7 (4.6) | 27.7 (2.6) | 30.9 (3.3) | 24.5 (2.6) |
| Bundle spacing (μm) | ASD | 53.8 (7.2) | 57.6 (7.0) | 53.6 (7.0) | 61.7 (8.0) | 63.9 (8.9) | 34.1 (3.1) |
| | CTL | 56.3 (5.4) | 58.0 (9.2) | 54.7 (10.9) | 58.4 (9.3) | 69.3 (12.6) | 34.7 (5.3) |
| Bundle width (μm) | ASD | 9.9 (1.3) | 9.2 (0.8) | 9.3 (2.0) | 9.4 (2.1) | 8.2 (1.4) | 7.9 (1.1) |
| | CTL | 9.8 (1.2) | 8.8 (1.3) | 8.2 (1.3) | 9.7 (1.0) | 9.2 (1.5) | 8.2 (0.9) |
| FA | ASD | 0.081 (0.036) | 0.073 (0.034) | 0.086 (0.043) | 0.084 (0.052) | 0.087 (0.043) | 0.11 (0.07) |
| | CTL | 0.092 (0.016) | 0.113 (0.035) | 0.100 (0.025) | 0.090 (0.027) | 0.112 (0.050) | 0.076 (0.036) |
| MD | ASD | 0.00025 (0.00012) | 0.00029 (0.00013) | 0.00021 (0.00010) | 0.00019 (0.000086) | 0.00019 (0.00010) | 0.00018 (0.00011) |
| | CTL | 0.00019 (0.00005) | 0.00016 (0.00001) | 0.00017 (0.00005) | 0.00013 (0.00003) | 0.00011 (0.00002) | 0.00018 (0.00007) |
| Θ_{rad} (rad) | ASD | 0.80 (0.23) | 0.91 (0.26) | 0.84 (0.18) | 0.80 (0.23) | 0.79 (0.16) | 0.78 (0.24) |
| | CTL | 0.54 (0.12) | 0.56 (0.24) | 0.75 (0.19) | 0.59 (0.25) | 0.76 (0.32) | 0.91 (0.36) |
| D_{\perp} | ASD | 0.00012 (0.000084) | 0.00019 (0.00011) | 0.00011 (0.00005) | 0.00010 (0.00005) | 0.00009 (0.00006) | 0.00010 (0.00005) |
| | CTL | 0.00007 (0.00004) | 0.00007 (0.00003) | 0.00010 (0.00006) | 0.00006 (0.00002) | 0.00005 (0.00002) | 0.00010 (0.00006) |

Table 5.4. Measured variables within each region of cerebral cortex (mean and SD) for both ASD and control cases.

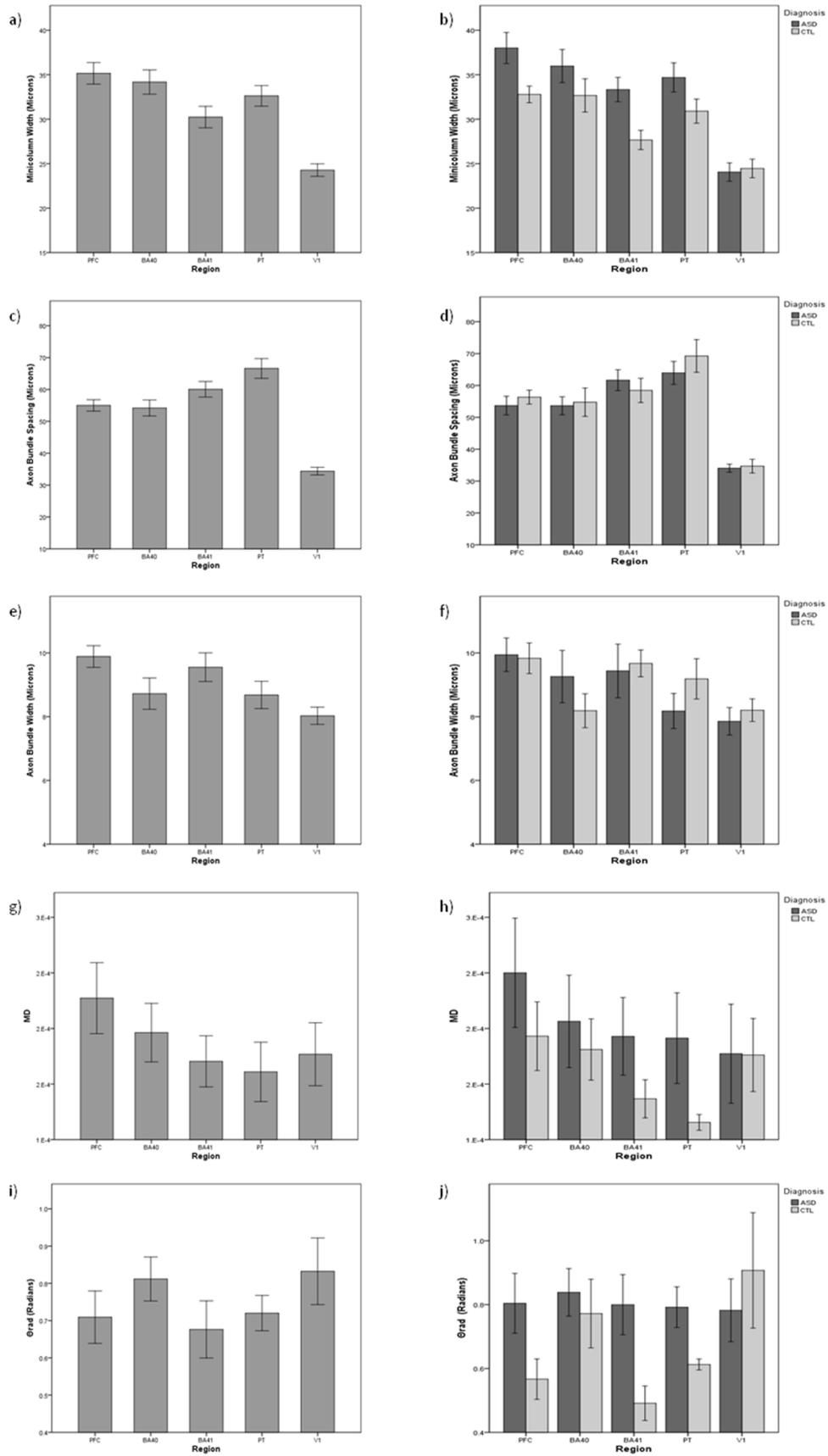


Figure 5.2. Average values for histology measures and measures of cortical diffusion for each cortical region for both groups together (a, c, e, g and i) and for ASD and control groups separately (b, d, f, h and j). Error bars represent the standard error of the mean.

Regional Differences according to Cortex Type

Regions were grouped according to whether they were primary sensory regions (BA41 and V1), unimodal association cortex (PT) or heteromodal association cortex (BA9 and BA40, again BA11 was excluded due to a number of missing values) in order to test the suggestion that microstructural differences in ASD may be specific to certain types of cortex (Buxhoeveden et al., 2006). A repeated-measures ANOVA with 'cortex type' as the within-subjects factor and diagnosis as the between-subjects factor was conducted for each measure individually. A significant effect of cortex type was observed in minicolumn width ($p < 0.001$), bundle spacing ($p < 0.001$) and MD ($p = 0.023$). Post-hoc investigation of these main effects revealed significant differences in minicolumn width and bundle spacing between all types of cortex and significantly higher MD in heteromodal association areas than unimodal association cortex (Figure 5.3) (as only three post-hoc tests were carried out for each measurement type, no corrections for multiple comparisons are necessary). No interactions between cortex type and diagnosis were observed (all $p > 0.1$) (Table 5.5).

Although regional differences are found across both ASD and control groups, this does not address the question of whether these regional differences are attenuated in ASD compared to controls. In order to investigate this, for each of the measures of histology and cortical diffusion, the increase in values going from one cortex type to the next (in a hierarchical manner) was calculated. These values were investigated using repeated-measures ANOVAs with 'cortical hierarchy' as the within-subjects factor and diagnosis as the between-subjects factor. A significant effect of cortical hierarchy was seen for bundle spacing ($p < 0.001$), MD ($p < 0.001$) and D_{\perp} ($p = 0.001$). In addition both MD and D_{\perp}

| | Histology Measures | | | Measures of Cortical Diffusion | | | |
|--------------------------------------|-------------------------------------|--------------------------------|-----------------------------|--------------------------------|---------------------------------|-----------------------------|--------------------------------|
| | Minicolumn Width | Bundle Spacing | Bundle Width | FA | MD | Θ_{rad} | D_{\perp} |
| Effect of cortex type | F(1.2, 11.0) = 61.45 P < 0.001** | F(2,20) = 36.37 P < 0.001** | F(2,20) = 0.86 P = 0.438 | F(2,16) = 0.04 P = 0.959 | F(1.1,9.0) = 7.24 P = 0.023* | F(2,16) = 0.30 P = 0.744 | F(1.2,9.7) = 4.02 P = 0.069 |
| Cortex type by diagnosis interaction | F(1.2, 11.0) = 0.72 P = 0.442 | F(2,20) = 0.58 P = 0.569 | F(2,20) = 1.25 P = 0.309 | F(2,16) = 2.04 P = 0.162 | F(1.1,9.0) = 1.41 P = 0.272 | F(2,16) = 0.25 P = 0.785 | F(1.2,9.7) = 0.82 P = 0.410 |
| Effect of diagnosis | F(1,9) = 4.70 P = 0.058 | F(1,10) = 0.51 P = 0.493 | F(1,10) = 0.32 P = 0.584 | F(1,8) = 0.01 P = 0.929 | F(1,8) = 0.89 P = 0.374 | F(1,8) = 3.91 P = 0.083 | F(1,8) = 0.98 P = 0.352 |

Table 5.5. Overall effects determined by repeated-measures ANOVAs are reported in the first row (effect of cortex type)

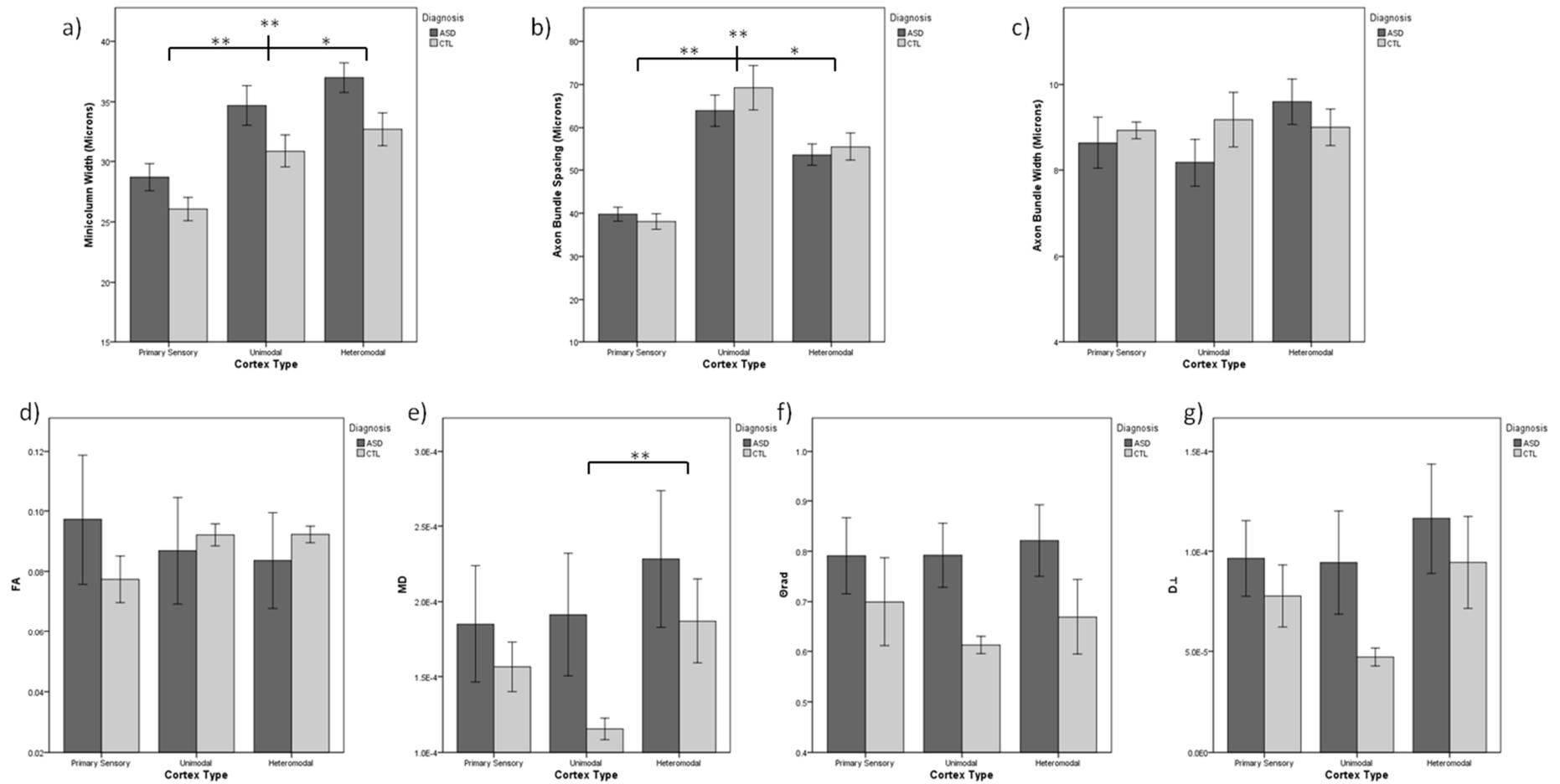


Figure 5.3. Average values for histology measures (top row) and measures of cortical diffusion (bottom row) for each type of cortex (primary sensory, unimodal association and heteromodal association) for ASD and control groups separately. Error bars represent the standard error of the mean.

| | Histology Measures | | | Measures of Cortical Diffusion | | | |
|---------------------------------------------|----------------------------|------------------------------|-----------------------------|--------------------------------|-------------------------------|----------------------------|-------------------------------|
| | Minicolumn Width | Bundle Spacing | Bundle Width | FA | MD | Θ_{rad} | D_{\perp} |
| Effect of cortical hierarchy | F(1, 9) = 4.49 P=0.063 | F(1,10) = 32.80 P<0.001** | F(1,10) = 0.53 P = 0.482 | F(1,10) = 0.15 P = 0.705 | F(1,8) = 49.27 P<0.001** | F(1,8) = 0.73 P = 0.417 | F(1,8) = 24.81 P = 0.001** |
| Cortical hierarchy by diagnosis interaction | F(1, 9) = 0.045 P=0.836 | F(1,10) = 0.57 P=0.468 | F(1,10) = 1.33 P = 0.276 | F(1,10) = 1.31 P = 0.285 | F(1,8) = 16.05 P = 0.004** | F(1,8) = 0.33 P = 0.581 | F(1,8) = 6.94 P = 0.030* |
| Effect of diagnosis | F(1,9) = 4.90 P = 0.054 | F(1,10) = 0.62 P = 0.450 | F(1,10) = 1.10 P = 0.320 | F(1,8) = 2.29 P = 0.169 | F(1,8) = 0.10 P = 0.760 | F(1,8) = 0.19 P = 0.676 | F(1,8) = 0.01 P = 0.924 |

Table 5.6. Overall effects determined by repeated-measures ANOVAs are reported in the first row (effect of cortical hierarchy).

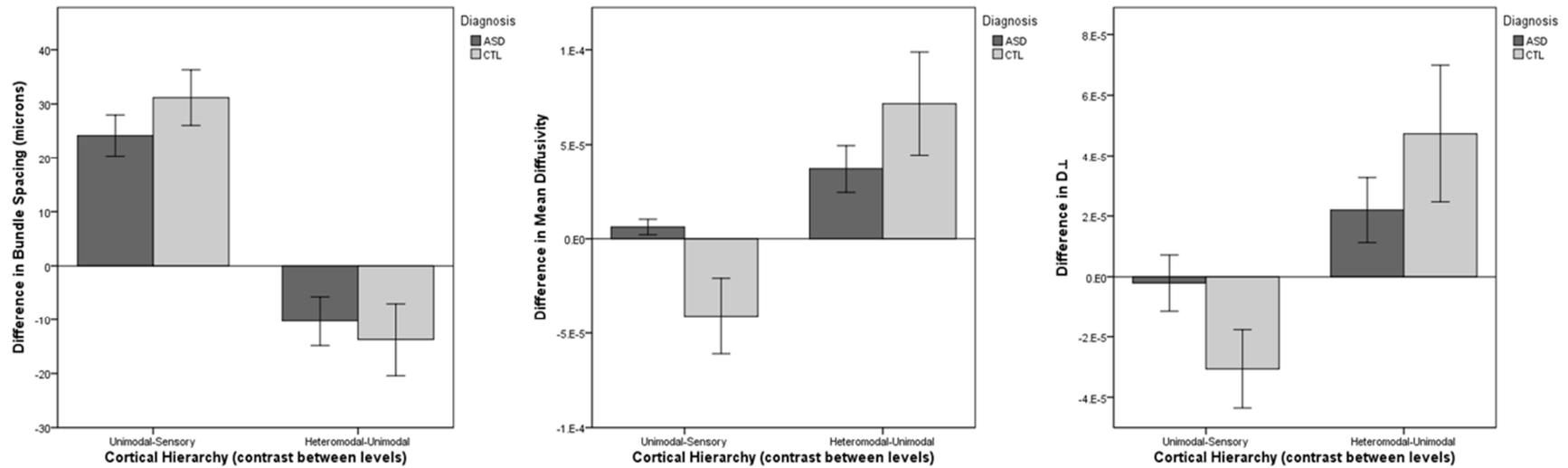


Figure 5.4. Difference in histology measures and measures of cortical diffusion between one level in the cortical hierarchy and the next, for both ASD and control cases shown separately.

showed an interaction between cortical hierarchy and diagnosis ($p=0.004$ and $p=0.030$ respectively). No significant effects of diagnosis were observed (all $p>0.05$) (Table 5.6). Visual inspection of the data revealed that smaller differences in values were seen between different cortical hierarchies in ASD when compared to controls, and, that for the measures of cortical diffusion, this difference seemed to be greatest when comparing data from primary sensory regions with unimodal association cortex (Figure 5.4).

Relationship between histology and DTI

No correlations between histology and DTI measurements were seen for all cortical regions considered together across both ASD and control groups, although a trend towards a negative correlation was observed between bundle spacing and D_{\perp} ($r=-0.225$, $p=0.084$). There was also a trend towards a correlation between bundle spacing and FA ($r=0.215$, $p=0.099$) and bundle spacing and MD ($r=-0.234$, $p=0.072$). When ASD and control groups were considered separately a significant positive correlation was seen between bundle spacing and FA ($r=0.421$, $p=0.029$) in controls only.

In order to determine whether the ratio between axon bundle width and axon bundle spacing varies across cortical regions the percentage of the bundle spacing occupied by the width of the bundles themselves was calculated. BA11 was excluded from this analysis as axon bundle measurements were unavailable for three out of six ASD cases. A repeated-measures ANOVA with region as the within-subjects factor and diagnosis as the between-subjects factor revealed a significant main effect of region ($F(4,40)=22.5$, $p<0.001$) but no effect of diagnosis ($F(1,10)=0.007$, $p=0.937$) and no interaction between region and diagnosis ($F(4,40)=0.753$, $p=0.562$). Therefore correlations were investigated in each region individually. Due to missing values for BA11 in a number of cases BA11 was not included in these investigations.

A significant positive correlation was seen between bundle spacing and Θ_{rad} for controls in BA41 ($r=0.888$, $p=0.044$) as well as a trend towards a positive correlation between axon bundle width and Θ_{rad} for controls in BA41 ($r=0.841$, $p=0.074$) and PT ($r=0.876$, $p=0.052$). Although not significant for ASD cases, for a number of regions the relationships of a priori interest (i.e. between axon bundle width and both Θ_{rad} and D_{\perp}) seemed to be in the opposite direction to that seen in controls cases. Therefore Fisher's r-to-z transformation was used to calculate whether the relationships were significantly different between ASD and control groups. This transforms correlation coefficients so they are normally distributed and so can be compared using a Z-test.

Axon Bundle Width and Θ_{rad}

Correlations in ASD and controls were compared using Fisher's r-to-z transformation revealing a significantly different relationship in PT ($z=-1.93$, $p=0.027$) and a trend towards a different relationship in BA41 ($z=-1.51$, $p=0.066$) (Table 5.7).

| Region | Diagnosis | Pearson correlation | | Fisher's r-to-z transformation | |
|--------|-----------|-------------------------|-------------|--------------------------------|--------|
| | | r | p | Z | p |
| BA9 | ASD | $r = 0.187$ $n = 6$ | $p = 0.722$ | -0.29 | 0.386 |
| | CTL | $r = 0.427$ $n = 5$ | $p = 0.474$ | | |
| BA40 | ASD | $r = -0.451$ $n = 6$ | $p = 0.369$ | -1.06 | 0.145 |
| | CTL | $r = 0.446$ $n = 5$ | $p = 0.452$ | | |
| BA41 | ASD | $r = -0.152$ $n = 6$ | $p = 0.774$ | -1.51 | 0.066 |
| | CTL | $r = 0.841$ $n = 5$ | $p = 0.074$ | | |
| PT | ASD | $r = -0.380$ $n = 6$ | $p = 0.457$ | -1.93 | 0.027* |
| | CTL | $r = 0.876$ $n = 5$ | $p = 0.052$ | | |
| V1 | ASD | $r = 0.583$ $n = 6$ | $p = 0.225$ | -0.40 | 0.345 |
| | CTL | $r = -0.204$ $n = 4$ | $p = 0.796$ | | |

Table 5.7. Comparison of the relationship between axon bundle width and Θ_{rad} for each cortical region, between control and ASD cases.

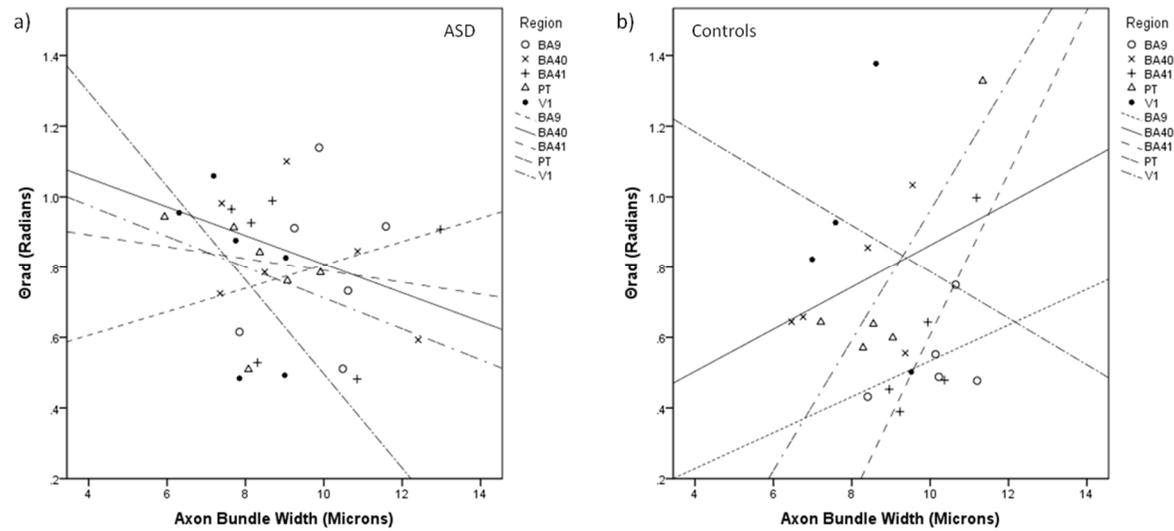


Figure 5.5. Least squares regression lines for the relationship between axon bundle width and Θ_{rad} for each cortical region in a) ASD and b) control cases

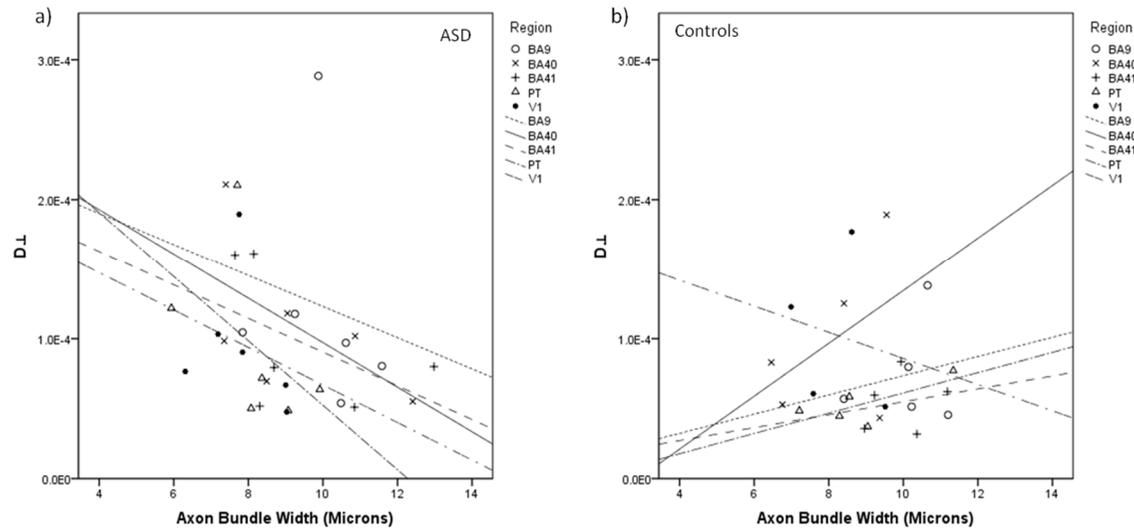


Figure 5.6. Least squares regression lines for the relationship between axon bundle width and D_{\perp} for each cortical region in a) ASD and b) control cases

Axon Bundle Width and D_{\perp}

Visual inspection of the data reveals a clear tendency for the relationship to be negative in ASD cases but, with the exception of BA40, positive in control cases. Closer inspection of the individual regions in the ASD cases revealed that ASD05 appeared to be an outlier in a number of areas and exclusion of this subject increased the strength of the relationships.

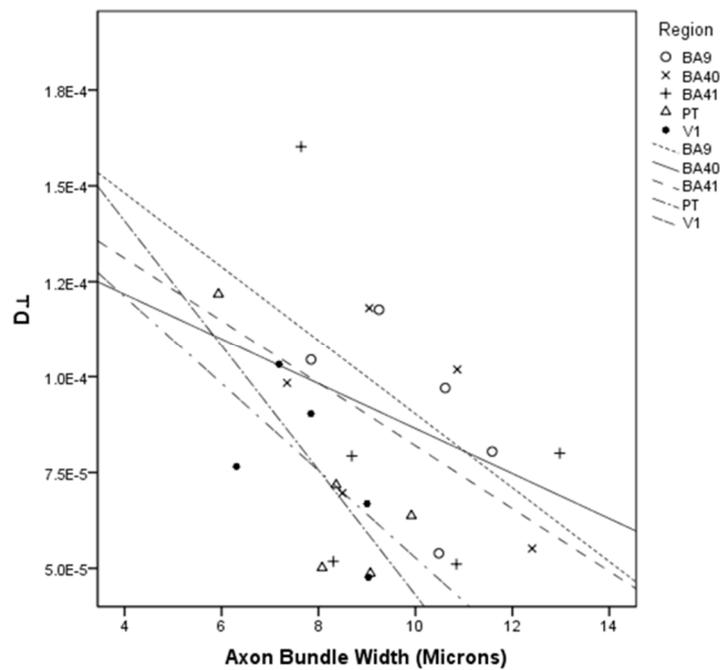


Figure 5.7. Least squares regression line for the relationship between axon bundle width and D_{\perp} for each cortical region in ASD cases after exclusion of ASD05.

Correlations in ASD and controls were compared using Fisher's r-to-z transformation, with ASD and control groups showing a significantly different relationship in PT ($z=-2.03$, $p=0.021$) though differences in other regions did not reach significance (all $p>0.1$) (Table 5.8).

| Region | Diagnosis | Pearson correlation | | Fisher's r-to-z transformation | |
|--------|-----------|---------------------|-----------|--------------------------------|--------|
| | | r | p | Z | p |
| BA9 | ASD | r = -0.565 n = 5 | p = 0.321 | -0.83 | 0.203 |
| | CTL | r = 0.190 n = 5 | p = 0.760 | | |
| BA40 | ASD | r = -0.460 n = 5 | p = 0.436 | -0.99 | 0.161 |
| | CTL | r = 0.455 n = 5 | p = 0.441 | | |
| BA41 | ASD | r = -0.403 n = 5 | p = 0.501 | -0.62 | 0.268 |
| | CTL | r = 0.195 n = 5 | p = 0.753 | | |
| PT | ASD | r = -0.812 n = 5 | p = 0.095 | -2.03 | 0.021* |
| | CTL | r = 0.716 n = 5 | p = 0.174 | | |
| V1 | ASD | r = -0.626 n = 5 | p = 0.259 | -0.45 | 0.326 |
| | CTL | r = -0.178 n = 4 | p = 0.822 | | |

Table 5.8. Comparison of the relationship between axon bundle width and D_{\perp} for each cortical region, between control and ASD cases.

Relationship between histology measures

Bundle spacing was found to correlate with both minicolumn width ($r= 0.479$, $p<0.001$) (Figure 5.8a) and bundle width ($r=0.401$, $p=0.001$) (Figure 5.8b). Both these relationships were significant in ASD and control groups separately (bundle spacing and minicolumn width: ASD $r= 0.510$, $p= 0.003$ control $r= 0.572$, $p<0.001$; bundle spacing and bundle width: ASD $r= 0.457$, $p=0.008$ control $r= 0.355$, $p=0.034$). In addition, a trend correlation was observed between minicolumn width and bundle width ($r=0.216$, $p=0.079$) (Figure 5.8c) although this did not reach significance in either ASD ($r=0.212$, $p=0.245$) or control subjects ($r=0.266$, $p=0.122$) alone.

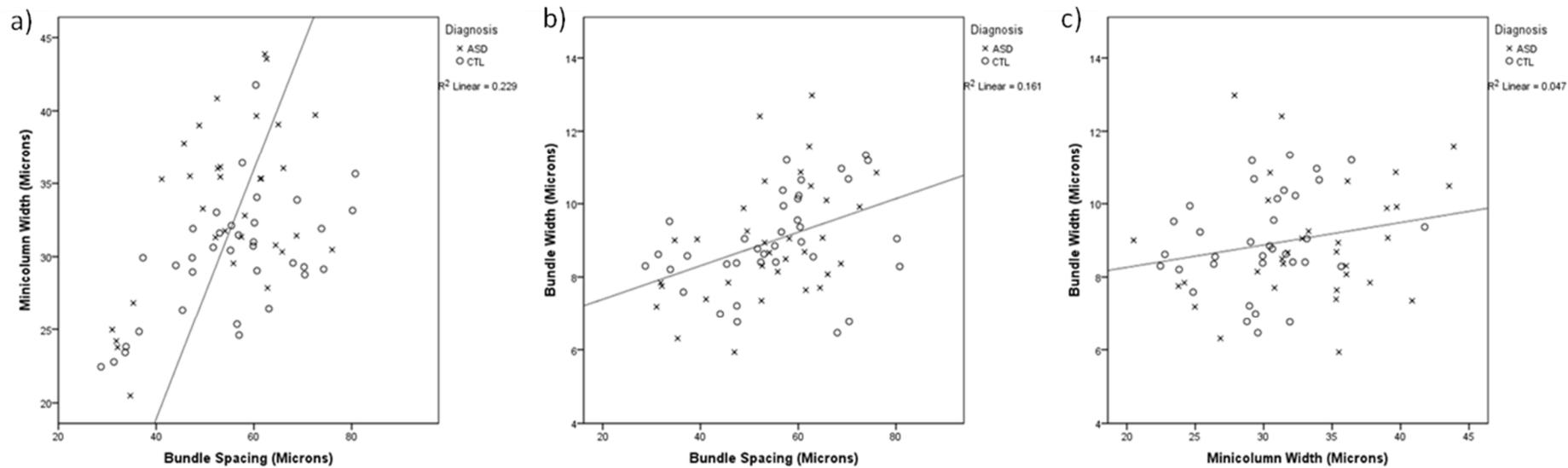


Figure 5.8. Least squares regression lines for the relationship between histology measures.

Relationship with demographic measures

An independent samples t-test revealed that the control group were significantly older than the ASD group ($t(10)=-4.006$, $p=0.002$) (Figure 5.9). However, when considering ASD and control groups separately, age was not found to correlate with any of the histological or cortical diffusion measures of interest (all $p>0.1$). For this reason, and because the effects of age might be different in ASD and control groups, age was not factored into the analyses.

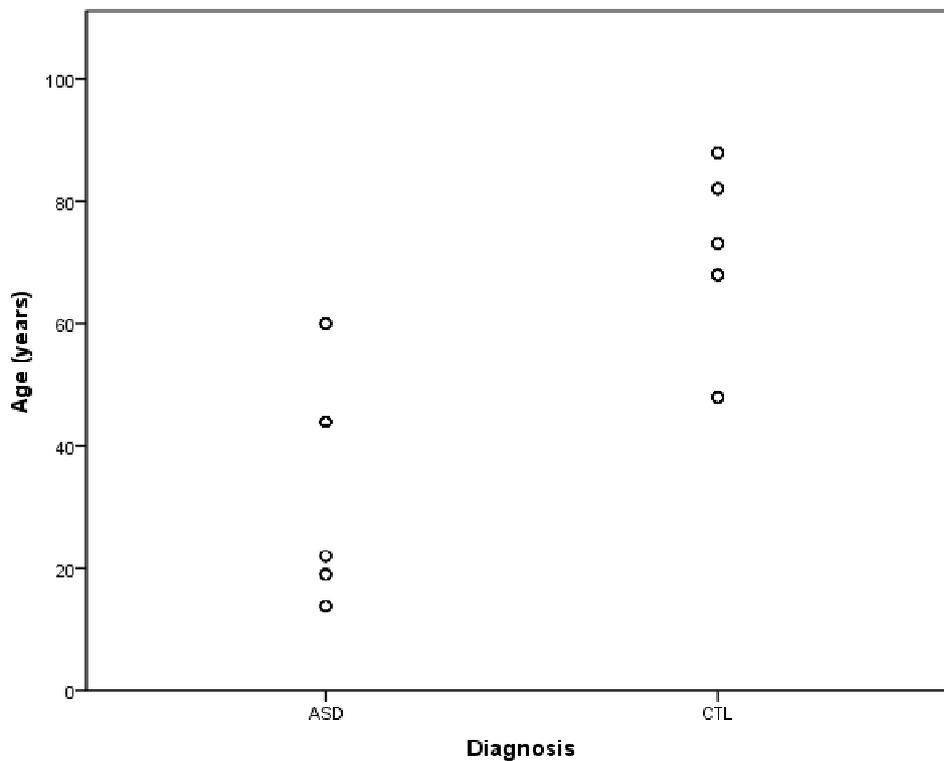


Figure 5.9. Distribution of ages of ASD and control cases

No significant correlations were observed between post-mortem interval (PMI) and mean measures of cortical diffusion (calculated across all ROIs for each subject) (all $p>0.05$). Scan interval (SI), on the other hand, was found to correlate with both mean MD ($r=0.935$, $p<0.001$) and mean D_{\perp} ($r=0.939$, $p<0.001$). However, this was found to be primarily due to one ASD case with a particularly long SI (ASD05), as if this case was

excluded no significant correlations were seen between SI and measures of cortical diffusion (all $p > 0.01$). Most findings were unaffected by exclusion of this case, although it did reduce the significance of some results. In particular exclusion of ASD05 resulted in a trend effect of region on MD values ($F(1.83, 12.84) = 3.227$, $p = 0.076$) and a trend interaction between cortical hierarchy and diagnosis on D_{\perp} ($F(1, 7) = 5.13$, $p = 0.058$). In addition, there was only a trend difference in the relationship between axon bundle width and Θ_{rad} in the PT between ASD and control cases ($z = -1.72$, $p = 0.085$). However, some of this reduction in significance may have been due to the reduction in the number of data points also.

Brain weight was not found to correlate with measures of minicolumn width, bundle spacing or axon bundle width (all $p > 0.2$).

Discussion

Diagnostic differences

The primary aim of the present study was to investigate differences between ASD and control subjects. Diagnostic differences in minicolumn width were detected, with increased minicolumn width being seen in ASD, as reported previously (Chapter 3). Although no diagnostic differences were found in the remaining histology measures (width and spacing of the axon bundles), it is possible that this could be due to the age of the subjects included in this study. The ASD subjects (14-60 years) were significantly younger than the control subjects (48-88 years), with very little overlap in the distribution (Figure 5.9). Although not statistically significant, previous work (Chapter 3) has found a tendency for axon bundles to be wider in young ASD cases and older control cases, and

so the distribution of ages in the present study may have minimised any differences between diagnostic groups.

Given that correlations between axon bundle width and measures of cortical diffusion were not detected in the present study, and that diagnostic differences were not apparent for axon bundle width, it is perhaps not surprising that diagnostic differences in measures of cortical diffusion were not found in this data set. Although this means it is not clear whether the measurements of cortical diffusion would be expected to be increased or decreased in ASD, there is also a confounding factor from the age difference between the two groups. Measures of diffusion anisotropy in grey matter have been found to decline with aging (Bhagat and Beaulieu, 2004; Nusbaum et al., 2001) and one study has reported increases in MD with age (Jeon et al., 2012), although several other studies suggest the amount of diffusion occurring in the cortex does not change with age (Gideon et al., 1994; Helenius et al., 2002). This suggests that work is still needed to clarify the relationship between ageing and standard measures of diffusion (FA and MD), let alone the novel measures used in the present study (Θ_{rad} and D_{\perp}), and so it would be premature to conclude that the present results reflect a true lack of diagnostic differences in these measures.

Regional Differences

The present study found regional differences in both the cyto- and myelo-architecture consistent with previous descriptions of the cortex (DeFelipe, 2005; Von Economo and Koskinas, 1925). As was found in the data from the MS cases and previous work (Casanova et al., 2008; Veluw et al., 2012), PFC showed the widest minicolumns and axon bundles, while V1 showed the narrowest. When cortical regions were grouped according to their cortex type (i.e. primary sensory, unimodal or heteromodal association

cortex) differences were only seen for minicolumn width and bundle spacing. For minicolumn width, primary sensory cortex showed the narrowest bundles, with wider bundles being seen in unimodal association cortex and the widest bundles being seen in heteromodal association cortex, which is consistent with previous findings (Chance, 2006). Given the strong correspondence between minicolumn width and bundle spacing (Casanova et al., 2008) it is surprising that bundle spacing did not show the same pattern of increase progressing through levels of association cortex. Instead the widest bundle spacing was found in unimodal association cortex, followed by heteromodal association cortex and then primary sensory cortex. Unimodal association cortex in the present study consisted of the planum temporale, an area which is known to display hemispheric asymmetries in minicolumn width (Chance et al., 2006) and so could be expected to show asymmetries in bundle spacing (though this has never been investigated). Although it is possible that larger values in the left hemisphere could lead to a higher overall average value for the PT it is unlikely that that is the explanation in the current study as both right and left PT show larger values for bundle spacing than does heteromodal association cortex. It is possible, however, that there is something unique or unusual about the PT that means the bundle spacing in this area is not representative of unimodal association cortex. Therefore future work should investigate bundle spacing in more examples of unimodal association cortex.

The present study did not find evidence for the absence of cortical differentiation, as was suggested by a previous study (Voineagu et al., 2011). However, the difference between cortical hierarchies did appear smaller in ASD cases than in controls, suggesting attenuated cortical differentiation consistent with the findings of Ziats and Rennert (2013). Buxhoeveden et al. (2006) have suggested that differences between subjects with ASD and controls may be more pronounced in the higher order association cortices but

this was not found in the present study, as reflected in the finding of no significant interactions between cortex type and diagnosis. Axon bundle spacing seemed to show similar diagnostic differences for all types of cortex, although diagnostic differences in measures of cortical diffusivity seemed to be more pronounced in unimodal association cortex (Figure 5.3). Again, as unimodal association cortex is represented only by the PT it would be interesting to investigate whether a similar pattern was seen when looking at another example of unimodal association cortex.

Given the sensitivity of measures of cortical diffusion to regional differences in the MS data it is surprising that regional differences were only detected by MD in the present data set. There are several possible explanations for this. Although we did not detect reduced cortical differentiation in ASD in the histology measures investigated, there are other components of the cortical architecture that may influence cortical diffusion and may show reduced cortical differentiation in their organisation. For example, dendritic organisation has been suggested to contribute to cortical anisotropy (McNab et al., 2013) and has also been found to be altered in ASD (Hutsler and Zhang, 2010; Raymond et al., 1995; Wass, 2011). It would be interesting for future studies to investigate whether dendritic organisation in ASD differs from controls in a regionally dependant manner. The lack of an interaction between cortical region and diagnosis suggests that these measures of cortical diffusion do not show regional differentiation in controls either. Measures of cortical diffusion have been observed to change with age (Bhagat and Beaulieu, 2004; Nusbaum et al., 2001), although with the exception of MD this has not been systematically investigated with respect to cortical region. Age related changes in MD have been shown to vary with cortical region, with only some areas showing significant changes (Jeon et al., 2012), suggesting that a similar pattern could also occur in other measures of cortical diffusion. It is possible therefore, that age-related changes in

measures of FA, Θ_{rad} and D_{\perp} may have been more pronounced in some areas in such a way that the differences between the areas investigated in the present study were minimised. It would be interesting for future research to establish the regional pattern of age-related changes in measures of cortical diffusivity.

Of the measures of cortical diffusivity, the present study found regional differences for MD only, with controls showing the highest MD in PFC, followed by BA40, V1, BA41 and finally PT. This is consistent with the only other study to have examined MD at a regional level in adults in a similar age range (present study: 44-88 years; Jeon et al. (2012): 65.7 ± 7.2 years). Although this study examined MD at a gyral level, identifying the gyri that most closely match the regions investigated in the present study revealed a similar pattern of results with the superior frontal gyrus (corresponding to PFC) showing the highest MD followed by the supramarginal gyrus (BA40), pericalcarine cortex (containing V1) and finally the superior temporal gyrus (which includes both BA41 and PT) (Figure 5.10).

The present study found that for ASD subjects MD was highest in PFC followed by BA40, BA41 and PT and finally by V1, which does not quite correspond with the findings of Jeon et al. (2012) for either young or older controls. Although it is difficult to make a direct comparison, as Jeon et al. (2012) only report results on the gyral level, which does not correspond perfectly to the ROIs used in the present study (particularly in the case of the STG which covers both BA41 and PT), this suggests there may be some differences between MD in ASD and controls which the present study was unable to detect, possibly due to the small sample size.

When the ROIs are grouped by cortex type a significant effect of cortex type is seen on values of MD, along with a trend effect on D_{\perp} . This suggests that D_{\perp} is perhaps not

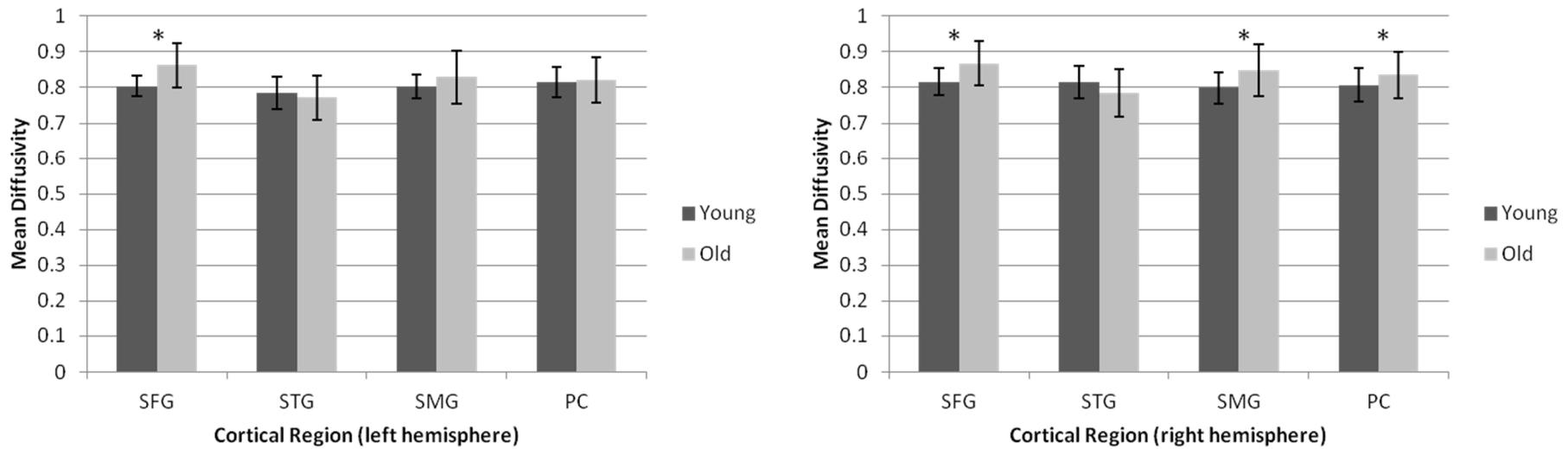


Figure 5.10. Cortical mean diffusivities measured on a gyral level for both young (age: 22.6 ± 4.6 years) and old (age: 65.7 ± 7.2 years) controls. Results were reported on a gyral level for left and right hemispheres separately. Taken from Jeon et al (2012). * indicates significant differences in MD between age groups at $p < 0.05$. SFG=superior frontal gyrus; STG=superior temporal gyrus; SMG=supramarginal gyrus and PC=pericalcarine cortex.

sensitive enough to detect the more subtle differences present between different regions of cortex, but may be sensitive enough to detect the larger differences between different cortex types. These two measures of cortical diffusion seem to also be sensitive to cortical differentiation measured in terms of the difference in these measures between different levels in the cortical hierarchy (Table 5.6). Further, both show a significant interaction between cortical hierarchy and diagnosis, which seems to be consistent with attenuated, but not absent, cortical differentiation in ASD (Figure 5.4) as suggested by Ziats and Rennert (2013). Comparison of Figures 5.2 and 5.3 suggests that, in contrast to the suggestion of Buxhoeveden et al. (2006) that differences might be minimal in primary sensory regions and more pronounced in higher order association cortices, the largest differences between ASD and control subjects may be in unimodal association cortex. However, it is still worth considering the possibility that this could be something unique to the PT rather than all unimodal association cortices. In addition, considering the pattern of age-related changes in MD (Figure 5.10) (Jeon et al., 2012), it is possible that the significant increases in MD in controls in both heteromodal association areas and pericalcarine cortex could have minimised differences between ASD and control subjects in these areas and not in unimodal association cortex. Future studies should aim to clarify these issues.

Relationship between histology and DTI

One aim of the present study was to characterise the relationship between DTI and histology for ASD and control cases. It is surprising that the present study was not able to detect the correlations of a priori interest, i.e. those between axon bundle width and both Θ_{rad} and D_{\perp} . This may have been due to the small sample size used in the present study (just five control brains compared to nine MS brains in the previous study) although data

points were collected from more regions for each brain. However, another possible reason for this relates to the uncertainty surrounding which parameter(s) of axon bundle width were actually driving the correlations (e.g. increased numbers of axons, increased g-ratio, wider spacing of axons). For example, in MS demyelination may have led to artificially smaller axon bundles with a correspondingly lower g-ratio, and so a stronger correlation between axon bundle width and thickness of myelin sheathes than would be seen in healthy control tissue. Therefore, although a correlation may still exist between the thickness of the myelin sheath and cortical diffusion measures in control tissue, we are not able to observe this by measuring axon bundle width. This possibility could also explain why, for the ASD group, the relationships between axon bundle width and both Θ_{rad} and D_{\perp} go predominantly in the opposite direction to those seen in controls. As it is unlikely that the relationship between cortical diffusion and histology is diagnosis dependent, the altered relationship in ASD may instead reflect a difference in the relationship between axon bundle width and one of the other properties of the axon bundle (e.g. number of axons, axon density etc). Therefore, more work is needed in order to determine which aspect(s) of axonal bundle organisation are giving rise to these differences.

Relationship between histology measures

The present finding of a correlation between spacing of the minicolumns based on cell bodies and axon bundles is consistent with what is known about the structure of the minicolumn (Buxhoeveden and Casanova, 2002; Mountcastle, 1997) and previous work comparing the two measurements (Casanova et al., 2008).

Very few studies have been conducted to investigate the organisation of axonal bundles in the cortex, and indeed how different parameters of axonal bundle organisation relate to

one another. It is interesting to note that there seems to be a positive relationship between axon bundle spacing and axon bundle width across both the MS and ASD and control data sets (reaching significance in the MS data and showing a trend relationship in the ASD and control data). However, it is perhaps more interesting that this is not a straightforward relationship as the percentage of the bundle spacing which is occupied by the width of the bundles themselves shows regional variation. However, it is unclear whether the variation in axon bundle width is due to different numbers of individual fibers within the bundle, different widths of the axons themselves, changes in the g-ratio affecting the thickness of the myelin sheath around the axons or changes in the density of packing of the same number of fibers. It would be interesting for future studies to clarify which of these parameters are changing and whether different parameters are responsible for the relationship with axon bundle spacing and the regional differences.

Limitations

As with the MS data reported in the previous chapter (Chapter 4) the present study has been limited to investigating six regions of the cortex (PFC, BA40, BA41, PT, V1, and BA11, although BA11 was not included in the final analysis due to several missing values) due to the time-consuming nature of the manual steps involved in both histological processing and image analysis. It would be interesting for future studies to extend this investigation to a greater number of cortical regions.

Recent work has demonstrated superior diffusion tractography, particularly in the presence of crossing fibers, using DTI data collected with the steady-state free precession (DW-SSFP) technique as compared to the spin echo technique used in the present study (McNab et al., 2009; Miller et al., 2012). Therefore it is possible that use of the DW-

SSFP technique would result in better quality diffusion measures in the cortex also, allowing the relationships reported in the MS data and here to be investigated further.

Unfortunately the current study is limited by the small sample size available, a problem shared by many studies using post-mortem tissue. In the present study this small sample size may have resulted in insufficient statistical power to detect relationships between histology measures and measures of cortical diffusion. In addition, due to brain tissue availability it was not possible to match the ASD and control subjects for age, which may have made it more difficult to detect any diagnostic differences. However, the MS data provides preliminary evidence for a relationship between cortical diffusion and measures of cortical architecture. In addition, the ASD and control data suggest possible attenuation of cortical differentiation. These are both interesting findings that should be investigated in a larger data set.

Post-mortem tissue is known to show altered diffusion properties (Miller et al., 2011) and studies have demonstrated reductions in both FA and MD in white matter, e.g. (Miller et al., 2011; Schmierer et al., 2008). Some studies have found a dependence of diffusivity measures on post-mortem interval (PMI) (D'Arceuil and de Crespigny, 2007; Miller et al., 2011) although others have suggested these remain relatively constant after fixation (Kim et al., 2009). However, these effects have mainly been investigated in white matter and have been much less studied in grey matter. It is worth noting that tissue volumetric change due to fixation (i.e. shrinkage) stabilizes within a few weeks (Quester and Schröder, 1997) and most of the cases in this study had been fixed for little more than the three years that Dyrby et al (2011) suggest is the length of the initial period of stable scan interval (SI – time between death and scan). Investigation of the effect of both PMI and SI on measures of cortical diffusion revealed relationships only with scan interval that seemed to be dependent on the one case with a particularly long scan interval. Removal

of this case had no effect on the overall pattern of results. This suggests that care should be taken when investigating cases with an extremely long SI.

Conclusion

The present study suggests measures of cortical diffusion (in particular MD, and to a lesser extent D_{\perp}) are sensitive to regional differences in terms of type of cortex (i.e., primary sensory, unimodal or heteromodal association cortex). In addition, these measures may prove useful for investigating the degree of differentiation between cortical regions in disorders such as ASD where reduced differentiation has been suggested (Voineagu et al., 2011; Ziats and Rennert, 2013). Although the present study was not able to replicate previous findings of relationships between axon bundle width and Θ_{rad} and D_{\perp} , the finding of significantly different relationships between these measures in ASD and control subjects suggests this is an area requiring further investigation to determine which specific components of axonal bundle width are differing between ASD and control subjects and underlying these relationships.

Chapter 6:

Conclusions

The aim of this work was to investigate the neuropathology of the socio-cognitive network in ASD, focusing on four key cortical areas involved in processes known to be affected in ASD. Primary auditory cortex was included due to the high rate of auditory abnormalities in ASD (Klintwall et al., 2011). The planum temporale (PT) is involved in language, which is known to be affected in ASD. BA11 and BA40 are both important in theory of mind (Abu-Akel and Shamay-Tsoory, 2011; Brink et al., 2011; Carrington and Bailey, 2009; Sabbagh, 2004; Völlm et al., 2006).

This work combined analysis of both in-vivo and post-mortem measurements to investigate structural differences at both the macro- and micro-scopic levels. This involved the application of established methods of measuring minicolumn width, to areas not previously investigated in ASD, in a much larger cohort than previous studies, as well as the development and application of novel methods for investigation of cortical diffusion. In doing so, two important themes have become apparent in this work: the importance of developmental trajectory and regional differences. These will be discussed in more detail below.

Age

Age was found to be an important factor throughout these studies, with measures taken both in-vivo and post-mortem showing an altered relationship with age in ASD. In-vivo volumetric measurements showed a positive relationship between age and volume of both BA40 and BA41 in subjects with ASD, that was absent (in the case of BA40) or

negative (in the case of BA41) in controls. This age dependence may have contributed to the overall finding of no volumetric difference between the groups across the whole age range. Diagnostic differences in minicolumn width also seemed to be age dependent, with the finding of wider minicolumns in ASD seeming to be primarily driven by large differences in the young cases, with decreasing differences seen with increasing age.

These findings fit with the idea of an altered developmental trajectory suggested by Courchesne (Courchesne et al., 2011; Courchesne and Pierce, 2005), and also previous findings suggesting age dependent differences in neuron size (Jacot-Descombes et al., 2012; Kemper and Bauman, 1998; Kemper and Bauman, 2002) and cortical thickness (Ecker et al., 2014; Zielinski et al., 2014). In particular the minicolumnar findings fit with Courchesne's findings of more pronounced structural differences at early ages, which then normalise. However, an important difference between Courchesne's volumetric findings and the current minicolumn findings is that Courchesne suggests the 'normalisation' of brain volume in ASD is achieved through the controls catching up. In contrast, we see a negative relationship between age and minicolumn width in both groups, but that the rate of decrease is faster in ASD subjects.

Regional Differences

The studies presented here report two important results with regard to regional differences. Firstly, differences were found across all types of cortex studied, including primary sensory regions, in terms of both volumetric relationships with age and minicolumnar differences. This is important as it contradicts previous suggestions that the pathology is restricted to higher order association areas while primary sensory areas are unaffected (Buxhoeveden et al., 2006). In addition, it provides evidence of

anatomical differences which may be associated with the, previously under-recognised, high incidence of sensory abnormalities in ASD (Klintwall et al., 2011).

Secondly, regional variation was found to be preserved in ASD although not as pronounced as in controls. This was found across both measures of histology (minicolumn and axon bundle measurements) and some measures of cortical diffusion. This argues against the idea of ASD being characterised by a lack of regional differentiation within the cortex. Although regional differentiation did not seem to be as pronounced in ASD as control cases, the lack of any region by diagnoses interactions argues against this being a result of different patterns of cortical differences in the two groups. That is, although the differences between regions seemed to be smaller in ASD, the widest minicolumns were found in BA40, and the narrowest in BA41, for both ASD and control cases.

Together these results suggest that alterations in the minicolumnar structure are a widespread feature of ASD, affecting many different types of cortex to similar degrees, and therefore suggests minicolumnar abnormalities may be a unifying feature of ASD, underlying all aspects of the symptomatology seen in ASD.

ASD severity

The present work detected relationships between regional brain volumes and measures of ASD severity (ADI-R and ADOS-G scores) in the corresponding domains (i.e. BA41 and social and communication measures, and BA11 and social ability). Surprisingly, such a relationship was not detected using post-mortem measures of microstructure. Although such correlations have not been reported before for measures of cortical microstructure (although an association has been found between Purkinje cell density in the cerebellum and use of social eye contact (Skefos et al., 2014)), it would be surprising if alterations in

minicolumn width, which may affect the fundamental processing of information, were not related to symptom severity. The lack of associations in the present study may be due to the large number of cases for which this information was not available.

MRI vs. Histology

The present work found volumetric differences using MRI in grey matter volume of BA11, and in the left occipito-parietal region. However, histological approaches were able to detect differences in BA11, BA41 and PT, suggesting that microstructural changes may exist in the absence of macroscopic ones. This therefore suggests that histological investigation of microstructure may be more sensitive to ASD-related differences than MRI-based gross volumetric measures.

We were able to show a good correspondence between MRI measures of cortical diffusion and histological measures of cortical architecture in a series of MS brains, suggesting a possible role for high-resolution DTI scans to provide information at both the macro- and microscopic levels of the cortex. However, more complicated relationships were seen when extending this investigation to a set of ASD and control scans, which suggested that the relationship between axon bundle width and cortical diffusion may be driven by another parameter which contributes to axon bundle width, but that may be altered in ASD without affecting the overall axon bundle width. This shows how using a combination of MRI and histology approaches may be more effective than either alone, and how information from one may be used to inform understanding of the other.

In addition, while perhaps not as informative as to the exact nature of the structural changes in the cortex, measures of cortical diffusivity were shown to be a useful measure, sensitive to cortical differentiation. Therefore, with further development of this

technique to establish its validity in-vivo, this could be a useful approach to investigating regional differences and even cortical development, in healthy aging and pathology, across the lifespan.

Interpretation

Evidence from cytoarchitectural studies has suggested that wider spacing of minicolumns will result in more independent functioning of the individual minicolumn (Chance et al., 2012; Harasty et al., 2003; Seldon, 1981b). This would facilitate greater discrimination and less generalisation of stimuli and provide a potential neural basis for ‘weak central coherence’. As wider minicolumns are seen across a range of cortical areas, not just primary sensory ones, such reduced generalisation could underlie ASD symptomatology in all three domains. Inability to generalise stimuli will impair extraction of salient features from a situation, and so will impact upon both communication and social interaction (Gustafsson, 1997). In addition, such a focus on individual features of a stimulus may lead to over-awareness of changes to such features (for example the placement of an object within a room or a particular stage of a routine), and so give rise to restricted and repetitive behaviours (Gustafsson, 1997). Such reduced integration across stimuli would also explain the observed increased temporal resolution in ASD, discussed in Chapter 2.

In addition, the current finding of increased minicolumn width is consistent with previous suggestions of an alteration in the excitation-inhibition balance. Computational models of minicolumnar development support the idea that decreased inhibition would lead to wider minicolumns (Gustafsson, 2004). Therefore, an altered ratio of excitation:inhibition could act as an underlying cause of differences in minicolumnar width, and may co-exist alongside such structural changes. This would therefore be able

to explain the observation of behaviours consistent with reduced cortical inhibition in ASD, as well as the high incidence of seizures (Tannan et al., 2008; Tommerdahl et al., 2007; Tommerdahl et al., 2008; Vattikuti and Chow, 2010).

Limitations and Future Work

Although the present work provides the largest investigation into minicolumnar abnormalities in ASD, as well as a novel method for investigation of differences in cortical diffusion, there are several limitations. Apart from the study of minicolumnar differences, the other studies unfortunately had quite small sample sizes, which may have limited the ability to detect subtle diagnostic differences. However, both of these studies find interesting results that future studies should look into investigating in larger populations.

Given that differences in minicolumn width appear to be most pronounced at younger ages it is a limitation of the study into cortical diffusion in ASD that we had few young ASD cases, and that all the control cases were much older. This may be why diagnostic differences were not detected in either the histological or cortical diffusion measures. It would again be interesting for future studies to extend this work, in order to investigate the sensitivity of cortical diffusion to diagnostic differences, when these are detectable in the histology measures.

Although the study of minicolumn abnormalities included a large number of cases, once variables such as gender, presence of seizures and specific age groups are considered, the number of cases in each group becomes rather small, limiting the ability to detect any differences between these groups. Therefore future studies should try to use larger homogeneous groups

One major limitation of post-mortem studies of minicolumn width in ASD in general is the lack of consistency between methods used. For example, the present study used frozen tissue, sectioned at 30 μ m, whereas previous studies have used celloidin embedded tissue, sectioned at anywhere between 35 and 500 μ m. As discussed in Chapters 3 and 5, these are all factors which could influence the measurements of minicolumn width and so prevent the comparison of values across studies. In addition, there is a lack of consistency between which measures have been reported previously, with some studies finding a decrease in minicolumn width (Casanova et al., 2006a), some in both minicolumn width and neuropil space (Casanova et al., 2002b), and some finding a significant effect of diagnosis for neuropil space only (Casanova et al., 2006b).

It would be interesting for future work to go on to investigate the differences in the relationship between cortical diffusion measures and axon bundle width in control and ASD groups. In particular it would be interesting to use electron microscopy to investigate whether individual components of the axon bundle are varying between diagnostic groups, for example whether the g-ratio differs between groups. This would not be unprecedented as Zikopoulos and Barbas (2010) reported an increased g-ratio in ASD in orbital-frontal cortex. It would be interesting to build on this greater characterisation of axon bundles in both controls and ASD in order to better understand the correlation between cortical diffusion and axon bundle width, and why this is absent in ASD.

Finally, the interpretation of the functional implications of the wider minicolumns seen in ASD depends on observations made in control tissue. In particular, these observations are largely based on observations of reduced dendritic overlap between minicolumns when they are more widely spaced in one hemisphere than the other. This means this relationship may not extend to differences in minicolumn width within an area even

within controls, and it is not known whether this relationship would hold true in disorders such as ASD. Therefore, future work should assess the validity of this assumption in ASD, either by detailed assessment of dendritic arborisation using electron microscopy, or by assessment of density of staining with dendritic markers such as MAP2.

Conclusion

ASD is clearly a complex neurodevelopmental disorder with a heterogeneous presentation, both behaviourally and structurally. However, the current work has added to a growing literature showing that these structural findings become more consistent when considered within a developmental framework, suggesting a period of accelerated growth during early childhood, followed by a period of attenuated growth or even decline, resulting in more subtle differences in adulthood. In addition, we have been able to show that differences in developmental trajectory can be observed across the cortex, and do not seem to be confined to 'higher-order' association areas. Moreover, the altered processing suggested by wider minicolumns provides a potential neural basis for the bias towards local, rather than global, processing seen in ASD.

References

- Abell F, Krams M, Ashburner J, Passingham R, Friston K, Frackowiak R, Happé F, Frith CD, Frith U. (1999): The neuroanatomy of autism: a voxel-based whole brain analysis of structural scans. *NeuroReport* 10(8):1647-51.
- Abrams DA, Lynch CJ, Cheng KM, Phillips J, Supekar K, Ryali S, Uddin LQ, Menon V. (2013): Underconnectivity between voice-selective cortex and reward circuitry in children with autism. *Proceedings of the National Academy of Sciences* 110(29):12060-12065.
- Abu-Akel A, Shamay-Tsoory S. (2011): Neuroanatomical and neurochemical bases of theory of mind. *Neuropsychologia* 49(11):2971-2984.
- Adams N, Jarrold C. (2012): Inhibition in Autism: Children with Autism have Difficulty Inhibiting Irrelevant Distractors but not Prepotent Responses. *Journal of Autism and Developmental Disorders* 42(6):1052-1063.
- Agam Y, Joseph RM, Barton JJS, Manoach DS. (2010): Reduced cognitive control of response inhibition by the anterior cingulate cortex in autism spectrum disorders. *NeuroImage* 52(1):336-347.
- Alexander AL, Lee JE, Lazar M, Boudos R, DuBray MB, Oakes TR, Miller JN, Lu J, Jeong E-K, McMahon WM and others. (2007): Diffusion tensor imaging of the corpus callosum in Autism. *NeuroImage* 34(1):61-73.
- Alexander I, Cowey A, Walsh V. (2005): The right parietal cortex and time perception: back to Critchley and the Zeigler phenomenon. *Cognitive Neuropsychology* 22(3-4):306-315.
- Allely CS, Gillberg C, Wilson P. Neurobiological abnormalities in the first few years of life in individuals later diagnosed with Autistic Spectrum Disorder: A review of recent data. *Behavioural Neurology*.
- Amaral DG, Schumann CM, Nordahl CW. (2008): Neuroanatomy of autism. *Trends in Neurosciences* 31(3):137-145.
- Ameis SH, Fan J, Rockel C, Voineskos AN, Lobaugh NJ, Soorya L, Wang AT, Hollander E, Anagnostou E. (2011): Impaired Structural Connectivity of Socio-Emotional Circuits in Autism Spectrum Disorders: A Diffusion Tensor Imaging Study. *PLoS ONE* 6(11):e28044.
- Anderson JS, Druzgal TJ, Froehlich A, DuBray MB, Lange N, Alexander AL, Abildskov T, Nielsen JA, Cariello AN, Cooperrider JR and others. (2011): Decreased Interhemispheric Functional Connectivity in Autism. *Cerebral Cortex* 21(5):1134-1146.
- Andersson JL, Jenkinson M, Smith S. 2007. Non-linear registration, aka Spatial normalisation. www.fmrib.ox.ac.uk/analysis/techrep.
- Anwander, A., Pampel, A., Knosche, T.R., 2010. In vivo measurement of cortical anisotropy by diffusion-weighted imaging correlates with cortex type. *Proc. Intl. Soc. Mag. Reson. Med* 18, 109.
- Ashburner J, Friston KJ. (2000): Voxel-Based Morphometry—The Methods. *NeuroImage* 11(6):805-821.
- Ashburner J, Friston KJ. (2001): Why Voxel-Based Morphometry Should Be Used. *NeuroImage* 14(6):1238-1243.
- Assaf M, Jagannathan K, Calhoun VD, Miller L, Stevens MC, Sahl R, O'Boyle JG, Schultz RT, Pearlson GD. (2010): Abnormal functional connectivity of default mode sub-networks in autism spectrum disorder patients. *NeuroImage* 53(1):247-256.
- Atladottir H, Gyllenberg D, Langridge A, Sandin S, Hansen S, Leonard H, Gissler M, Reichenberg A, Schendel D, Bourke J and others. (2014): The increasing prevalence of reported diagnoses of

- childhood psychiatric disorders: a descriptive multinational comparison. *European Child & Adolescent Psychiatry*:1-11.
- Avino TA, Hutsler JJ. (2010): Abnormal cell patterning at the cortical gray–white matter boundary in autism spectrum disorders. *Brain Research* 1360(0):138-146.
- Azmitia EC, Singh JS, Hou XP, Wegiel J. (2011): Dystrophic Serotonin Axons in Postmortem Brains from Young Autism Patients. *The Anatomical Record* 294(10):1653-1662.
- Badaruddin D, Andrews G, Bölte S, Schilmoeller K, Schilmoeller G, Paul L, Brown W. (2007): Social and Behavioral Problems of Children with Agenesis of the Corpus Callosum. *Child Psychiatry and Human Development* 38(4):287-302.
- Bagni C, Greenough WT. (2005): From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. *Nat Rev Neurosci* 6(5):376-387.
- Barazany, D., Assaf, Y., 2011. Visualization of Cortical Lamination Patterns with Magnetic Resonance Imaging. *Cerebral Cortex*.
- Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. (1995): Autism as a strongly genetic disorder: evidence from a British twin study. *Psychological Medicine* 25(01):63-77.
- Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Rutter M, Lantos P. (1998): A clinicopathological study of autism. *Brain* 121(5):889-905.
- Baird G, Simonoff E, Pickles A, Chandler S, Loucas T, Meldrum D, Charman T. Prevalence of disorders of the autism spectrum in a population cohort of children in South Thames: the Special Needs and Autism Project (SNAP). *The Lancet* 368(9531):210-215.
- Baker JP. (2013): Autism at 70 — Redrawing the Boundaries. *New England Journal of Medicine* 369(12):1089-1091.
- Banach R, Thompson A, Szatmari P, Goldberg J, Tuff L, Zwaigenbaum L, Mahoney W. (2009): Brief Report: Relationship Between Non-verbal IQ and Gender in Autism. *Journal of Autism and Developmental Disorders* 39(1):188-193.
- Barnea-Goraly N, Kwon H, Menon V, Eliez S, Lotspeich L, Reiss AL. (2004): White matter structure in autism: preliminary evidence from diffusion tensor imaging. *Biological Psychiatry* 55(3):323-326.
- Barnea-Goraly N, Lotspeich LJ, Reiss AL. (2010): Similar white matter aberrations in children with autism and their unaffected siblings: A diffusion tensor imaging study using tract-based spatial statistics. *Archives of General Psychiatry* 67(10):1052-1060.
- Barnea-Goraly N, Frazier TW, Piacenza L, Minshew NJ, Keshavan MS, Reiss AL, Hardan AY. (2014): A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 48(0):124-128.
- Baron-Cohen S. (1990): Autism: A Specific Cognitive Disorder of 'Mind-Blindness'. *International Review of Psychiatry* 2(1):81-90.
- Baron-Cohen S. 1996. *Mindblindness*: The MIT Press.
- Baron-Cohen S. 2003. *The Essential Difference*: Penguin.
- Baron-Cohen S, Jolliffe T, Mortimore C, Robertson M. (1997a): Another Advanced Test of Theory of Mind: Evidence from Very High Functioning Adults with Autism or Asperger Syndrome. *Journal of Child Psychology and Psychiatry* 38(7):813-822.

- Baron-Cohen S, Leslie AM, Frith U. (1985): Does the autistic child have a “theory of mind” ? *Cognition* 21(1):37-46.
- Baron-Cohen S, Richler J, Bisarya D, Gurunathan N, Wheelwright S. (2003): The systemizing quotient: an investigation of adults with Asperger syndrome or high-functioning autism, and normal sex differences. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 358(1430):361-374.
- Baron-Cohen S, Scott FJ, Allison C, Williams J, Bolton P, Matthews FE, Brayne C. (2009): Prevalence of autism-spectrum conditions: UK school-based population study. *The British Journal of Psychiatry* 194(6):500-509.
- Baron-Cohen S, Wheelwright S, Hill J, Raste Y, Plumb I. (2001): The “Reading the Mind in the Eyes” Test Revised Version: A Study with Normal Adults, and Adults with Asperger Syndrome or High-functioning Autism. *Journal of Child Psychology and Psychiatry* 42(2):241-251.
- Baron-Cohen S, Wheelwright S, Jolliffe, Therese. (1997b): Is There a "Language of the Eyes"? Evidence from Normal Adults, and Adults with Autism or Asperger Syndrome. *Visual Cognition* 4(3):311-331.
- Bauman M, Kemper TL. (1985): Histoanatomic observations of the brain in early infantile autism. *Neurology* 35(6):866.
- Bauman ML, Kemper TL. (1996): Observations on the Purkinje Cells in the Cerebellar Vermis in Autism. *Journal of Neuropathology & Experimental Neurology* 55(5):613.
- Bayliss A, Kritikos A. (2011): Brief Report: Perceptual Load and the Autism Spectrum in Typically Developed Individuals. *Journal of Autism and Developmental Disorders* 41(11):1573-1578.
- Beaulieu, C., 2002. The basis of anisotropic water diffusion in the nervous system – a technical review. *NMR in Biomedicine* 15, 435-455.
- Becker KG. (2004): The common variants/multiple disease hypothesis of common complex genetic disorders. *Medical Hypotheses* 62(2):309-317.
- Belmonte MK, Bourgeron T. (2006): Fragile X syndrome and autism at the intersection of genetic and neural networks. *Nat Neurosci* 9(10):1221-1225.
- Ben Bashat D, Kronfeld-Duenias V, Zachor DA, Ekstein PM, Hendler T, Tarrasch R, Even A, Levy Y, Ben Sira L. (2007): Accelerated maturation of white matter in young children with autism: A high b value DWI study. *NeuroImage* 37(1):40-47.
- Ben-Itzhak E, Ben-Shachar S, Zachor DA. (2013): Specific Neurological Phenotypes in Autism Spectrum Disorders Are Associated with Sex Representation. *Autism Res.*
- Berthold, C.H., Nilsson, I., Rydmark, M., 1983. Axon diameter and myelin sheath thickness in nerve fibres of the ventral spinal root of the seventh lumbar nerve of the adult and developing cat. *J Anat* 136, 483-508.
- Bhagat, Y.A., Beaulieu, C., 2004. Diffusion anisotropy in subcortical white matter and cortical gray matter: Changes with aging and the role of CSF-suppression. *Journal of Magnetic Resonance Imaging* 20, 216-227.
- Blumberg SJ, Bramlett MD, Kogan MD, Schieve LA, Jones JR, Lu MC. (2013): Changes in Prevalence of Parent-reported Autism Spectrum Disorder in School-aged U.S. Children: 2007 to 2011-2012. *National Health Statistics Reports* 65.
- Boddaert N, Chabane N, Gervais H, Good CD, Bourgeois M, Plumet MH, Barthélémy C, Mouren MC, Artiges E, Samson Y and others. (2004): Superior temporal sulcus anatomical abnormalities in childhood autism: a voxel-based morphometry MRI study. *NeuroImage* 23(1):364-369.

- Boger-Megiddo I, Shaw DW, Friedman S, Sparks B, Artru A, Giedd J, Dawson G, Dager S. (2006): Corpus Callosum Morphometrics in Young Children with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders* 36(6):733-739.
- Bölte S, Duketis E, Poustka F, Holtmann M. (2011): Sex differences in cognitive domains and their clinical correlates in higher-functioning autism spectrum disorders. *Autism* 15(4):497-511.
- Bonilha L, Cendes F, Rorden C, Eckert M, Dalgalarrrondo P, Li LM, Steiner CE. (2008): Gray and white matter imbalance – Typical structural abnormality underlying classic autism? *Brain and Development* 30(6):396-401.
- Bourgeron T. (2009): A synaptic trek to autism. *Current Opinion in Neurobiology* 19(2):231-234.
- Boyle CA, Boulet S, Schieve LA, Cohen RA, Blumberg SJ, Yeargin-Allsopp M, Visser S, Kogan MD. (2011): Trends in the Prevalence of Developmental Disabilities in US Children, 1997–2008. *Pediatrics* 127(6):1034-1042.
- Brambilla P, Hardan A, di Nemi SU, Perez J, Soares JC, Barale F. (2003): Brain anatomy and development in autism: review of structural MRI studies. *Brain Research Bulletin* 61(6):557-569.
- Brieber S, Neufang S, Bruning N, Kamp-Becker I, Remschmidt H, Herpertz-Dahlmann B, Fink GR, Konrad K. (2007): Structural brain abnormalities in adolescents with autism spectrum disorder and patients with attention deficit/hyperactivity disorder. *Journal of Child Psychology and Psychiatry* 48(12):1251-1258.
- Brink BP, Mork SJ, Van Der Valk P, Bo L. Grey Matter Pathology in Multiple Sclerosis. In: Comi G, Filippi M, Rovaris M, editors. *Normal-appearing White and Grey Matter Damage in Multiple Sclerosis*: Springer Milan; 2004. p. 101-9.
- Brink TT, Urton K, Held D, Kirilina E, Hofmann M, Klann-Delius G, Jacobs AM, Kuchinke L. (2011): The role of orbitofrontal cortex in processing empathy stories in 4-8 year-old children. *Frontiers in Psychology* 2.
- Brito AR, Vasconcelos MM, Dominques RC, Hygino da Cruz LCJ, LdeS R, Gasparetto EL, Calcada CA. (2009): Diffusion tensor imaging findings in school-aged autistic children. *J Neuroimaging* 19(4):337-43.
- Buchthal, F., Rosenfalck, A., 1966. Evoked action potentials and conduction velocity in human sensory nerves. *Brain Research* 3, v-vi, 1-122.
- Bueti D, Bahrami B, Walsh V. (2008): Sensory and Association Cortex in Time Perception. *Journal of Cognitive Neuroscience* 20(6):1054-1062.
- Buxhoeveden DP. 2012. Minicolumn size and human cortex. In: Hofman MA, Falk D, editors. *Evolution of the Primate Brain: From Neuron to Behavior*: Elsevier. p 219-235.
- Buxhoeveden DP, Casanova MF. (2002): The minicolumn hypothesis in neuroscience. *Brain* 125(5):935-951.
- Buxhoeveden DP, Semendeferi K, Buckwalter J, Schenker N, Switzer R, Courchesne E. (2006): Reduced minicolumns in the frontal cortex of patients with autism. *Neuropathology and Applied Neurobiology* 32(5):483-491.
- Buxhoeveden DP, Switala AE, Litaker M, Roy E, Casanova MF. (2001): Lateralization of Minicolumns in Human Planum temporale Is Absent in Nonhuman Primate Cortex. *Brain, Behavior and Evolution* 57(6):349-358.
- Calderoni S, Retico A, Biagi L, Tancredi R, Muratori F, Tosetti M. (2012): Female children with autism spectrum disorder: An insight from mass-univariate and pattern classification analyses. *NeuroImage* 59(2):1013-1022.

- Carlo CN, Stevens CF. (2011): Analysis of differential shrinkage in frozen brain sections and its implications for the use of guard zones in stereology. *The Journal of Comparative Neurology* 519(14):2803-2810.
- Carper RA, Moses P, Tigue ZD, Courchesne E. (2002): Cerebral Lobes in Autism: Early Hyperplasia and Abnormal Age Effects. *NeuroImage* 16(4):1038-1051.
- Carrington SJ, Bailey AJ. (2009): Are there theory of mind regions in the brain? A review of the neuroimaging literature. *Human Brain Mapping* 30(8):2313-2335.
- Carter A, Black D, Tewani S, Connolly C, Kadlec M, Tager-Flusberg H. (2007): Sex Differences in Toddlers with Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders* 37(1):86-97.
- Casanova M, El-Baz A, Mott M, Mannheim G, Hassan H, Fahmi R, Giedd J, Rumsey J, Switala A, Farag A. (2009): Reduced Gyral Window and Corpus Callosum Size in Autism: Possible Macroscopic Correlates of a Minicolumnopathy. *Journal of Autism and Developmental Disorders* 39(5):751-764.
- Casanova M, Kooten IJ, Switala A, Engeland H, Heinsen H, Steinbusch HM, Hof P, Trippe J, Stone J, Schmitz C. (2006a): Minicolumnar abnormalities in autism. *Acta Neuropathologica* 112(3):287-303.
- Casanova MF. (2006): Neuropathological and Genetic Findings in Autism: The Significance of a Putative Minicolumnopathy. *The Neuroscientist* 12(5):435-441.
- Casanova MF, Buxhoeveden DP, Brown C. (2002a): Clinical and Macroscopic Correlates of Minicolumnar Pathology in Autism. *Journal of Child Neurology* 17(9):692-695.
- Casanova MF, Buxhoeveden DP, Switala AE, Roy E. (2002b): Minicolumnar pathology in autism. *Neurology* 58(3):428-432.
- Casanova MF, Buxhoeveden DP, Switala AE, Roy E. (2002c): Neuronal Density and Architecture (Gray Level Index) in the Brains of Autistic Patients. *Journal of Child Neurology* 17(7):515-521.
- Casanova MF, El-Baz A, Kamat SS, Dombroski BA, Khalifa F, Elnakib A, Soliman A, Allison-McNutt A, Switala AE. (2013): Focal cortical dysplasias in autism spectrum disorders. *Acta Neuropathologica Communications* 1.
- Casanova MF, El-Baz A, Vanbogaert E, Narahari P, Switala A. (2010): A Topographic Study of Minicolumnar Core Width by Lamina Comparison between Autistic Subjects and Controls: Possible Minicolumnar Disruption due to an Anatomical Element In-Common to Multiple Laminae. *Brain Pathology* 20(2):451-458.
- Casanova, M.F., Konkachbaev, A.I., Switala, A.E., Elmaghraby, A.S., 2008. Recursive trace line method for detecting myelinated bundles: A comparison study with pyramidal cell arrays. *Journal of Neuroscience Methods* 168, 367-372.
- Casanova MF, van Kooten I, Switala AE, van Engeland H, Heinsen H, Steinbusch HWM, Hof PR, Schmitz C. (2006b): Abnormalities of cortical minicolumnar organization in the prefrontal lobes of autistic patients. *Clinical Neuroscience Research* 6(3-4):127-133.
- Castelli F, Frith C, Happé F, Frith U. (2002): Autism, Asperger syndrome and brain mechanisms for the attribution of mental states to animated shapes. *Brain* 125(8):1839-1849.
- Cauda F, Geda E, Sacco K, D'Agata F, Duca S, Geminiani G, Keller R. (2011): Grey matter abnormality in autism spectrum disorder: an activation likelihood estimation meta-analysis study. *Journal of Neurology, Neurosurgery & Psychiatry* 82(12):1304-1313.

- CDC. (2012): Prevalence of Autism Spectrum Disorders - Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008. *Surveillance Summaries* 61(SS03):1-19.
- Chakrabarti S, Fombonne E. (2005): Pervasive Developmental Disorders in Preschool Children: Confirmation of High Prevalence. *Am J Psychiatry* 162:1133-1141.
- Chance S, Casanova M, Switala A, Crow T, Esiri M. (2006): Minicolumn thinning in temporal lobe association cortex but not primary auditory cortex in normal human ageing. *Acta Neuropathologica* 111(5):459-464.
- Chance S, Sawyer E, Clover L, Wicinski B, Hof P, Crow T. (2012): Hemispheric asymmetry in the fusiform gyrus distinguishes *Homo sapiens* from chimpanzees. *Brain Structure and Function*:1-15.
- Chance SA. (2006): Subtle Changes in the Ageing Human Brain. *Nutrition and Health* 18(3):217-224.
- Chance, S.A., Casanova, M.F., Switala, A.E., Crow, T.J., 2006. Minicolumnar structure in Heschl's gyrus and planum temporale: Asymmetries in relation to sex and callosal fiber number. *Neuroscience* 143, 1041-1050.
- Chance SA, Casanova MF, Switala AE, Crow TJ. (2008): Auditory cortex asymmetry, altered minicolumn spacing and absence of ageing effects in schizophrenia. *Brain* 131(12):3178-3192.
- Chance SA, Clover L, Cousijn H, Currah L, Pettingill R, Esiri MM. (2011): Microanatomical Correlates of Cognitive Ability and Decline: Normal Ageing, MCI, and Alzheimer's Disease. *Cerebral Cortex* 21(8):1870-1878.
- Chance SA, Tzotzoli PM, Vitelli A, Esiri MM, Crow TJ. (2004): The cytoarchitecture of sulcal folding in Heschl's sulcus and the temporal cortex in the normal brain and schizophrenia: lamina thickness and cell density. *Neuroscience Letters* 367(3):384-388.
- Charman T, Baron-Cohen S. (1992): Understanding Drawings and Beliefs: a Further Test of the Metarepresentation Theory of Autism: a Research Note. *Journal of Child Psychology and Psychiatry* 33(6):1105-1112.
- Chatzopoulou, E., Miguez, A., Savvaki, M., Levasseur, G., Muzerelle, A., Muriel, M.-P., Goureau, O., Watanabe, K., Goutebroze, L., Gaspar, P., Zalc, B., Karageos, D., Thomas, J.-L., 2008. Structural Requirement of TAG-1 for Retinal Ganglion Cell Axons and Myelin in the Mouse Optic Nerve. *The Journal of Neuroscience* 28, 7624-7636.
- Cheng Y, Chou K-H, Chen IY, Fan Y-T, Decety J, Lin C-P. (2010): Atypical development of white matter microstructure in adolescents with autism spectrum disorders. *NeuroImage* 50(3):873-882.
- Cheon K-A, Kim Y-S, Oh S-H, Park S-Y, Yoon H-W, Herrington J, Nair A, Koh Y-J, Jang D-P, Kim Y-B and others. (2011): Involvement of the anterior thalamic radiation in boys with high functioning autism spectrum disorders: A Diffusion Tensor Imaging study. *Brain Research* 1417(0):77-86.
- Cherkassky VL, Kana RK, Keller TA, Just MA. (2006): Functional connectivity in a baseline resting-state network in autism. *NeuroReport* 17(16):1687-1690.
- Cheung C, Chua SE, Cheung V, Khong PL, Tai KS, Wong TKW, Ho TP, McAlonan GM. (2009): White matter fractional anisotropy differences and correlates of diagnostic symptoms in autism. *Journal of Child Psychology and Psychiatry* 50(9):1102-1112.
- Chow ML, Pramparo T, Winn ME, Barnes CC, Li H-R, Weiss L, Fan J-B, Murray S, April C, Belinson H and others. (2012): Age-Dependent Brain Gene Expression and Copy Number Anomalies in Autism Suggest Distinct Pathological Processes at Young Versus Mature Ages. *PLoS Genet* 8(3):e1002592.
- Chung MK, Robbins SM, Dalton KM, Davidson RJ, Alexander AL, Evans AC. (2005): Cortical thickness analysis in autism with heat kernel smoothing. *NeuroImage* 25(4):1256-1265.

- Cohen-Adad, J., Polimeni, J.R., Helmer, K.G., Benner, T., McNab, J.A., Wald, L.L., Rosen, B.R., Mainiero, C., 2012. T2* mapping and B0 orientation-dependence at 7T reveal cyto- and myeloarchitecture organization of the human cortex. *NeuroImage* 60, 1006-1014.
- Coleman P, Romano J, Lapham L, Simon W. (1985): Cell counts in cerebral cortex of an autistic patient. *Journal of Autism and Developmental Disorders* 15(3):245-255.
- Conturo TE, Williams DL, Smith CD, Gultepe E, Akbudak E, Minshew N. (2008): Neuronal fiber pathway abnormalities in autism: an initial MRI diffusion tensor tracking study of hippocampo-fusiform and amygdala-fusiform pathways. *J Int Neuropsychol Soc* 14(6):933-46.
- Courchesne E, Campbell K, Solso S. (2011a): Brain growth across the life span in autism: Age-specific changes in anatomical pathology. *Brain Research* 1380(0):138-145.
- Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, Chisum HJ, Moses P, Pierce K, Lord C and others. (2001): Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology* 57(2):245-254.
- Courchesne E, Mouton PR, Calhoun ME, et al. (2011b): Neuron number and size in prefrontal cortex of children with autism. *JAMA* 306(18):2001-2010.
- Courchesne E, Pierce K. (2005a): Brain overgrowth in autism during a critical time in development: implications for frontal pyramidal neuron and interneuron development and connectivity. *International Journal of Developmental Neuroscience* 23(2-3):153-170.
- Courchesne E, Pierce K. (2005b): Why the frontal cortex in autism might be talking only to itself: local over-connectivity but long-distance disconnection. *Current Opinion in Neurobiology* 15(2):225-230.
- Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, Morgan J. (2007): Mapping Early Brain Development in Autism. *Neuron* 56(2):399-413.
- Craig MC, Zaman SH, Daly EM, Cutter WJ, Robertson DMW, Hallahan B, Toal F, Reed S, Ambikapathy A, Brammer M and others. (2007): Women with autistic-spectrum disorder: magnetic resonance imaging study of brain anatomy. *The British Journal of Psychiatry* 191(3):224-228.
- Crino PB, Nathanson KL, Henske EP. (2006): The Tuberous Sclerosis Complex. *New England Journal of Medicine* 355(13):1345-1356.
- Croen L, Grether J, Hoogstrate J, Selvin S. (2002): The Changing Prevalence of Autism in California. *Journal of Autism and Developmental Disorders* 32(3):207-215.
- D'Arceuil, H., de Crespigny, A., 2007. The effects of brain tissue decomposition on diffusion tensor imaging and tractography. *NeuroImage* 36, 64-68.
- Da Costa S, van der Zwaag W, Marques JP, Frackowiak RSJ, Clarke S, Saenz M. (2011): Human Primary Auditory Cortex Follows the Shape of Heschl's Gyrus. *The Journal of Neuroscience* 31(40):14067-14075.
- Daymont C, Hwang W-T, Feudtner C, Rubin D. (2010): Head-Circumference Distribution in a Large Primary Care Network Differs From CDC and WHO Curves. *Pediatrics* 126(4):e836-e842.
- DeFelipe J. 2005. Reflections on the Structure of the Cortical Minicolumn. In: Casanova MF, editor. *Neocortical Modularity and the Cell Minicolumn*. New York: Nova Science Publishers, Inc. p 57-92.
- Di Rosa E, Crow TJ, Walker MA, Black G, Chance SA. (2009): Reduced neuron density, enlarged minicolumn spacing and altered ageing effects in fusiform cortex in schizophrenia. *Psychiatry Research* 166(2-3):102-115.

- Di Martino A, Kelly C, Grzadzinski R, Zuo X-N, Mennes M, Mairena MA, Lord C, Castellanos FX, Milham MP. (2011): Aberrant Striatal Functional Connectivity in Children with Autism. *Biological Psychiatry* 69(9):847-856.
- Dinstein I, Pierce K, Eyer L, Solso S, Malach R, Behrmann M, Courchesne E. (2011): Disrupted Neural Synchronization in Toddlers with Autism. *Neuron* 70(6):1218-1225.
- Dobrin PB. (1996): Effect of Histologic Preparation on the Cross-Sectional Area of Arterial Rings. *Journal of Surgical Research* 61(2):413-415.
- Dorph-Petersen K-A, Nyengaard JR, Gundersen HJG. (2001): Tissue shrinkage and unbiased stereological estimation of particle number and size. *Journal of Microscopy* 204(3):232-246.
- Douaud G, Smith S, Jenkinson M, Behrens T, Johansen-Berg H, Vickers J, James S, Voets N, Watkins K, Matthews PM and others. (2007): Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain* 130(9):2375-2386.
- Doulazmi M, Frédéric F, Lemaigre-Dubreuil Y, Hadj-Sahraoui N, Delhaye-Bouchaud N, Mariani J. (1999): Cerebellar purkinje cell loss during life span of the heterozygous Staggerer mouse (Rora+/Rorasg) is gender-related. *The Journal of Comparative Neurology* 411(2):267-273.
- Durkin MS, Maenner MJ, Meaney FJ, Levy SE, DiGuseppi C, Nicholas JS, Kirby RS, Pinto-Martin JA, Schieve LA. (2010): Socioeconomic inequality in the prevalence of autism spectrum disorder: evidence from a U.S. cross-sectional study. *PLoS One* 5(7).
- Dworzynski K, Ronald A, Bolton P, Happé F. (2012): How Different Are Girls and Boys Above and Below the Diagnostic Threshold for Autism Spectrum Disorders? *Journal of the American Academy of Child & Adolescent Psychiatry* 51(8):788-797.
- Dyment DA, Ebers GC, Dessa Sadovnick A. Genetics of multiple sclerosis. *The Lancet Neurology*. 2004;3(2):104-10.
- Dyrby TB, Baaré WFC, Alexander DC, Jelsing J, Garde E, Søgaard LV. An ex vivo imaging pipeline for producing high-quality and high-resolution diffusion-weighted imaging datasets. *Human Brain Mapping*. 2011;32(4):544-63.
- Ebisch SJH, Gallese V, Willems RM, Mantini D, Groen WB, Romani GL, Buitelaar JK, Bekkering H. (2011): Altered intrinsic functional connectivity of anterior and posterior insula regions in high-functioning participants with autism spectrum disorder. *Human Brain Mapping* 32(7):1013-1028.
- Ecker C, Marquand A, Mourão-Miranda J, Johnston P, Daly EM, Brammer MJ, Maltezos S, Murphy CM, Robertson D, Williams SC and others. (2010): Describing the Brain in Autism in Five Dimensions—Magnetic Resonance Imaging-Assisted Diagnosis of Autism Spectrum Disorder Using a Multiparameter Classification Approach. *The Journal of Neuroscience* 30(32):10612-10623.
- Ecker C, Ronan L, Feng Y, Daly E, Murphy C, Ginestet CE, Brammer M, Fletcher PC, Bullmore ET, Suckling J and others. (2013): Intrinsic gray-matter connectivity of the brain in adults with autism spectrum disorder. *Proceedings of the National Academy of Sciences* 110(32):13222-13227.
- Ecker C, Shahidiani A, Feng Y, Daly E, Murphy C, D’Almeida V, Deoni S, Williams SC, Gillan N, Gudbrandsen M and others. (2014): The effect of age, diagnosis, and their interaction on vertex-based measures of cortical thickness and surface area in autism spectrum disorder. *Journal of Neural Transmission*:1-14.
- Egaas B, Courchesne E, Saitoh O. (1995): REduced size of corpus callosum in autism. *Archives of Neurology* 52(8):794-801.
- El-Fishawy P, State MW. (2010): The Genetics of Autism: Key Issues, Recent Findings, and Clinical Implications. *Psychiatric Clinics of North America* 33(1):83-105.

- Elsabbagh M, Divan G, Koh Y-J, Kim YS, Kauchali S, Marcín C, Montiel-Nava C, Patel V, Paula CS, Wang C and others. (2012): Global Prevalence of Autism and Other Pervasive Developmental Disorders. *Autism Research* 5(3):160-179.
- Estes AM, Shaw DMM, Sparks BF, Friedman S, Giedd JN, Dawson G, Bryan M, Dager SR. (2011): Basal ganglia morphometry and repetitive behaviour in young children with autism spectrum disorder. *Autism Res* 4(3):212-220.
- Falter CM, Elliott MA, Bailey AJ. (2012a): Enhanced Visual Temporal Resolution in Autism Spectrum Disorders. *PLoS ONE* 7(3):e32774.
- Falter CM, Noreika V, Wearden JH, Bailey AJ. (2012b): More consistent, yet less sensitive: interval timing in autism spectrum disorders. *Q J Exp Psychol* 65(11):2093-107.
- Fatemi SH, Halt A, Realmuto G, Earle J, Kist D, Thuras P, Merz A. (2002): Purkinje Cell Size Is Reduced in Cerebellum of Patients with Autism. *Cellular and Molecular Neurobiology* 22(2):171-175.
- Fatterpekar, G.M., Naidich, T.P., Delman, B.N., Aguinaldo, J.G., Gultekin, S.H., Sherwood, C.C., Hof, P.R., Drayer, B.P., Fayad, Z.A., 2002. Cytoarchitecture of the Human Cerebral Cortex: MR Microscopy of Excised Specimens at 9.4 Tesla. *American Journal of Neuroradiology* 23, 1313-1321.
- Favorov OV, Kelly DG. (1994): Minicolumnar Organization within Somatosensory Cortical Segregates: I. Development of Afferent Connections. *Cerebral Cortex* 4(4):408-427.
- Fombonne E. (2009): Epidemiology of Pervasive Developmental Disorders. *Pediatr Res* 65(6):591-598.
- Foss-Feig J, Kwakye L, Cascio C, Burnette C, Kadivar H, Stone W, Wallace M. (2010): An extended multisensory temporal binding window in autism spectrum disorders. *Experimental Brain Research* 203(2):381-389.
- Frazier T, Keshavan M, Minshew N, Hardan A. (2012): A Two-Year Longitudinal MRI Study of the Corpus Callosum in Autism. *Journal of Autism and Developmental Disorders* 42(11):2312-2322.
- Frazier TW, Hardan AY. (2009): A Meta-Analysis of the Corpus Callosum in Autism. *Biological Psychiatry* 66(10):935-941.
- Freitag CM. (2006): The genetics of autistic disorders and its clinical relevance: a review of the literature. *Mol Psychiatry* 12(1):2-22.
- Freitag CM, Luders E, Hulst HE, Narr KL, Thompson PM, Toga AW, Krick C, Konrad C. (2009): Total Brain Volume and Corpus Callosum Size in Medication-Naïve Adolescents and Young Adults with Autism Spectrum Disorder. *Biological Psychiatry* 66(4):316-319.
- Friede, R.L., Beuche, W., 1985. Combined scatter diagrams of sheath thickness and fibre calibre in human sural nerves: changes with age and neuropathy. *Journal of Neurology, Neurosurgery & Psychiatry* 48, 749-756.
- Friede, R.L., Samorajski, T., 1967. Relation between the number of myelin lamellae and axon circumference in fibers of vagus and sciatic nerves of mice. *The Journal of Comparative Neurology* 130, 223-231.
- Friedman SD, Shaw DW, Artru AA, Dawson G, Petropoulos H, Dager SR. (2006): Gray and white matter brain chemistry in young children with autism. *Archives of General Psychiatry* 63(7):786-794.
- Friedman SD, Shaw DW, Artru AA, Richards TL, Gardner J, Dawson G, Posse S, Dager SR. (2003): Regional brain chemical alterations in young children with autism spectrum disorder. *Neurology* 60(1):100-107.

- Friston KJ, Frith CD, Frackowiak RSJ. (1993): Time-dependent changes in effective connectivity measured with PET. *Human Brain Mapping* 1(1):69-79.
- Frith U. 2003. *Autism: explaining the enigma*. Oxford: Blackwell Publishing.
- Frith U, Happé F. (1994): Autism: beyond “theory of mind”. *Cognition* 50(1–3):115-132.
- Gardella D, Hatton WJ, Rind HB, Rosen GD, von Bartheld CS. (2003): Differential tissue shrinkage and compression in the z-axis: implications for optical disector counting in vibratome-, plastic- and cryosections. *Journal of Neuroscience Methods* 124(1):45-59.
- Geier DA, Kern JK, King PG, Sykes LKG, M R. (2012): An evaluation of the role and treatment of elevated male hormones in autism spectrum disorders. *Acta Neurobiol Exp (Wars)* 72(1):1-17.
- Geschwind DH. (2011): Genetics of autism spectrum disorders. *Trends in Cognitive Sciences* 15(9):409-416.
- Gideon, P., Thomsen, C., Henriksen, O., 1994. Increased self-diffusion of brain water in normal aging. *Journal of Magnetic Resonance Imaging* 4, 185-188.
- Gillberg and C, Billstedt E. (2000): Autism and Asperger syndrome: coexistence with other clinical disorders. *Acta Psychiatrica Scandinavica* 102(5):321-330.
- Gilman Sarah R, Iossifov I, Levy D, Ronemus M, Wigler M, Vitkup D. (2011): Rare De Novo Variants Associated with Autism Implicate a Large Functional Network of Genes Involved in Formation and Function of Synapses. *Neuron* 70(5):898-907.
- Ginsberg MR, Rubin RA, Natowicz MR. (2013): Patterning of Regional Gene Expression in Autism: New Complexity. *Sci. Rep.* 3.
- Girgis RR, Minshew NJ, Melhem NM, Nutche JJ, Keshavan MS, Hardan AY. (2007): Volumetric alterations of the orbitofrontal cortex in autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 31(1):41-45.
- Giuliani NR, Calhoun VD, Pearlson GD, Francis A, Buchanan RW. (2005): Voxel-based morphometry versus region of interest: a comparison of two methods for analyzing gray matter differences in schizophrenia. *Schizophrenia Research* 74(2–3):135-147.
- Goldman S. (2013): Opinion: Sex, gender and the diagnosis of autism—A biosocial view of the male preponderance. *Research in Autism Spectrum Disorders* 7(6):675-679.
- Good CD, Johnsrude IS, Ashburner J, Henson RNA, Friston KJ, Frackowiak RSJ. (2001): A Voxel-Based Morphometric Study of Ageing in 465 Normal Adult Human Brains. *NeuroImage* 14(1):21-36.
- Gotts SJ, Simmons WK, Milbury LA, Wallace GL, Cox RW, Martin A. (2012): Fractionation of social brain circuits in autism spectrum disorders. *Brain* 135(9):2711-2725.
- Greimel E, Nehr Korn B, Schulte-Rüther M, Fink G, Nickl-Jockschat T, Herpertz-Dahlmann B, Konrad K, Eickhoff S. (2013): Changes in grey matter development in autism spectrum disorder. *Brain Structure and Function* 218(4):929-942.
- Groen WB, Buitelaar JK, van der Gaag RJ, Zwiers MP. (2011): Pervasive microstructural abnormalities in autism: a DTI study. *J Psychiatry Neurosci* 36(1):32-40.
- Groen WB, Zwiers MP, van der Gaag R-J, Buitelaar JK. (2008): The phenotype and neural correlates of language in autism: An integrative review. *Neuroscience & Biobehavioral Reviews* 32(8):1416-1425.
- Grondin S. (2010): Timing and time perception: A review of recent behavioral and neuroscience findings and theoretical directions. *Attention, Perception, & Psychophysics* 72(3):561-582.

- Gustafsson L. (1997): Inadequate cortical feature maps: A neural circuit theory of autism. *Biological Psychiatry* 42(12):1138-1147.
- Gustafsson L. (2004): Comment on "Disruption in the Inhibitory Architecture of the Cell Minicolumn: Implications for Autism". *The Neuroscientist* 10(3):189-191.
- Gutierrez G, Smalley S, Tanguay P. (1998): Autism in Tuberous Sclerosis Complex. *Journal of Autism and Developmental Disorders* 28(2):97-103.
- Guy, J., Ellis, E.A., Kelley, K., Hope, G.M., 1989. Spectra of G ratio, myelin sheath thickness, and axon and fiber diameter in the guinea pig optic nerve. *The Journal of Comparative Neurology* 287, 446-454.
- Hadjikhani N, Joseph RM, Snyder J, Tager-Flusberg H. (2006): Anatomical Differences in the Mirror Neuron System and Social Cognition Network in Autism. *Cerebral Cortex* 16(9):1276-1282.
- Hagberg KW, Jick H. (2010): Autism in the UK for Birth Cohorts 1988-2001. *Epidemiology* 21(3):426-427.
- Hagerman R, Hoem G, Hagerman P. (2010): Fragile X and autism: Intertwined at the molecular level leading to targeted treatments. *Molecular Autism* 1(12).
- Hall DA, Hart HC, Johnsrude IS. (2003): Relationships between Human Auditory Cortical Structure and Function. *Audiology and Neurotology* 8(1):1-18.
- Hallmayer J, Cleveland S, Torres A, et al. (2011): GENetic heritability and shared environmental factors among twin pairs with autism. *Archives of General Psychiatry* 68(11):1095-1102.
- Happé F. (1999): Autism: cognitive deficit or cognitive style? *Trends in Cognitive Sciences* 3(6):216-222.
- Happé F, Frith U. (2006): The Weak Coherence Account: Detail-focused Cognitive Style in Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders* 36(1):5-25.
- Happé F, Ronald A, Plomin R. (2006): Time to give up on a single explanation for autism. *Nat Neurosci* 9(10):1218-1220.
- Happé FGE. (1997): Central coherence and theory of mind in autism: Reading homographs in context. *British Journal of Developmental Psychology* 15(1):1-12.
- Harasty J, Seldon HL, Chan P, Halliday G, Harding A. (2003): The left human speech-processing cortex is thinner but longer than the right. *Laterality: Asymmetries of Body, Brain and Cognition* 8(3):247-260.
- Hardan AY, Girgis RR, Lacerda ALT, Yorbik O, Kilpatrick M, Keshavan MS, Minshew NJ. (2006): Magnetic Resonance Imaging Study of the Orbitofrontal Cortex in Autism. *Journal of Child Neurology* 21(10):866-871.
- Hardan AY, Libove RA, Keshavan MS, Melhem NM, Minshew NJ. (2009a): A Preliminary Longitudinal Magnetic Resonance Imaging Study of Brain Volume and Cortical Thickness in Autism. *Biological Psychiatry* 66(4):320-326.
- Hardan AY, Minshew NJ, Keshavan MS. (2000): Corpus callosum size in autism. *Neurology* 55(7):1033-1036.
- Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ. (2006): An MRI study of increased cortical thickness in autism. *Am J Psychiatry* 163:1290-1292.

- Hardan AY, Pabalan M, Gupta N, Bansal R, Melhem NM, Fedorov S, Keshavan MS, Minshew NJ. (2009b): Corpus callosum volume in children with autism. *Psychiatry Research: Neuroimaging* 174(1):57-61.
- Harrington DL, Boyd LA, Mayer AR, Sheltraw DM, Lee RR, Huang M, Rao SM. (2004): Neural representation of interval encoding and decision making. *Cognitive Brain Research* 21(2):193-205.
- Harrington DL, Haaland KY, Knight RT. (1998): Cortical Networks Underlying Mechanisms of Time Perception. *The Journal of Neuroscience* 18(3):1085-1095.
- Hartley S, Sikora D. (2009): Sex Differences in Autism Spectrum Disorder: An Examination of Developmental Functioning, Autistic Symptoms, and Coexisting Behavior Problems in Toddlers. *Journal of Autism and Developmental Disorders* 39(12):1715-1722.
- Hasan, K.M., Sankar, A., Halphen, C., Kramer, L.A., Brandt, M.E., Juranek, J., Cirino, P.T., Fletcher, J.M., Papanicolaou, A.C., Ewing-Cobbs, L., 2007. Development and organization of the human brain tissue compartments across the lifespan using diffusion tensor imaging. *NeuroReport* 18, 1735-1739.
- Hatton, W.J., Von Bartheld, C.S., 1999. Analysis of cell death in the trochlear nucleus of the chick embryo: Calibration of the optical disector counting method reveals systematic bias. *The Journal of Comparative Neurology* 409, 169-186.
- Hazlett HC, Poe MD, Gerig G, Smith RG, Piven J. (2006): Cortical Gray and White Brain Tissue Volume in Adolescents and Adults with Autism. *Biological Psychiatry* 59(1):1-6.
- Heidemann, R.M., Anwander, A., Knosche, T.R., Feiweier, T., F.Fasano, F., Pfeuffer, J., Turner, R., 2009. High resolution diffusion-weighted imaging showing radial anisotropy in the human cortex in vivo. *Proc. Intl. Soc. Mag. Reson. Med* 17, 1460.
- Helenius, J., Soinnie, L., Perkiö, J., Salonen, O., Kangasmäki, A., Kaste, M., Carano, R.A.D., Aronen, H.J., Tatlisumak, T., 2002. Diffusion-Weighted MR Imaging in Normal Human Brains in Various Age Groups. *American Journal of Neuroradiology* 23, 194-199.
- Hendry J, DeVito T, Gelman N, Densmore M, Rajakumar N, Pavlosky W, Williamson PC, Thompson PM, Drost DJ, Nicolson R. (2006): White matter abnormalities in autism detected through transverse relaxation time imaging. *NeuroImage* 29(4):1049-1057.
- Herbert MR, Ziegler DA, Deutsch CK, O'Brien LM, Lange N, Bakardjiev A, Hodgson J, Adrien KT, Steele S, Makris N and others. (2003): Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. *Brain* 126(5):1182-1192.
- Herbert MR, Ziegler DA, Makris N, Filipek PA, Kemper TL, Normandin JJ, Sanders HA, Kennedy DN, Caviness VS. (2004): Localization of white matter volume increase in autism and developmental language disorder. *Annals of Neurology* 55(4):530-540.
- Hinton SC, Meck WH. (2004): Frontal-striatal circuitry activated by human peak-interval timing in the supra-seconds range. *Cognitive Brain Research* 21(2):171-182.
- Hisaoka S, Harada M, Nishitani H, Mori K. (2001): Regional magnetic resonance spectroscopy of the brain in autistic individuals. *Neuroradiology* 43(6):496-498.
- Hollander E, Anagnostou E, Chaplin W, Esposito K, Haznedar MM, Licalzi E, Wasserman S, Soorya L, Buchsbaum M. (2005): Striatal Volume on Magnetic Resonance Imaging and Repetitive Behaviors in Autism. *Biological Psychiatry* 58(3):226-232.
- Holtmann M, Bölte S, Poustka F. (2007): Autism spectrum disorders: sex differences in autistic behaviour domains and coexisting psychopathology. *Developmental Medicine & Child Neurology* 49(5):361-366.

- Howlin P, Goode S, Hutton J, Rutter M. (2004): Adult outcome for children with autism. *Journal of Child Psychology and Psychiatry* 45(2):212-29.
- Hughes C, Russel J. (1993): Autistic children's difficulty with mental disengagement from an object: Its implications for theories of autism. *Developmental Psychology* 29(3):498-510.
- Hutsler JJ, Love T, Zhang H. (2007): Histological and Magnetic Resonance Imaging Assessment of Cortical Layering and Thickness in Autism Spectrum Disorders. *Biological Psychiatry* 61(4):449-457.
- Hutsler JJ, Zhang H. (2010): Increased dendritic spine densities on cortical projection neurons in autism spectrum disorders. *Brain Research* 1309(0):83-94.
- Hyde KL, Samson F, Evans AC, Mottron L. (2010): Neuroanatomical differences in brain areas implicated in perceptual and other core features of autism revealed by cortical thickness analysis and voxel-based morphometry. *Human Brain Mapping* 31(4):556-566.
- Jacobs, J.M., Love, S., 1985. Qualitative and Quantitative Morphology of Human Sural Nerve at Different Ages. *Brain* 108, 897-924.
- Jacot-Descombes S, Uppal N, Wicinski B, Santos M, Schmeidler J, Giannakopoulos P, Heinsein H, Schmitz C, Hof P. (2012): Decreased pyramidal neuron size in Brodmann areas 44 and 45 in patients with autism. *Acta Neuropathologica* 124(1):67-79.
- Jahanshahi M, Jones CRG, Dirnberger G, Frith CD. (2006): The Substantia Nigra Pars Compacta and Temporal Processing. *The Journal of Neuroscience* 26(47):12266-12273.
- Jancke L, Preis S, Steinmetz H. (1999): The relation between forebrain volume and midsagittal size of the corpus callosum in children. *NeuroReport* 10(4):2981-2985.
- Jäncke L, Staiger JF, Schlaug G, Huang Y, Steinmetz H. (1997): The relationship between corpus callosum size and forebrain volume. *Cerebral Cortex* 7(1):48-56.
- Jeon, T., Mishra, V., Uh, J., Weiner, M., Hatanpaa, K.J., White Iii, C.L., Zhao, Y.D., Lu, H., Diaz-Arrastia, R., Huang, H., 2012. Regional changes of cortical mean diffusivities with aging after correction of partial volume effects. *NeuroImage* 62, 1705-1716.
- Jeong J-W, Kumar AK, Sundaram SK, Chugani HT, Chugani DC. (2011): Sharp Curvature of Frontal Lobe White Matter Pathways in Children with Autism Spectrum Disorders: Tract-Based Morphometry Analysis. *American Journal of Neuroradiology* 32(9):1600-1606.
- Jespersen, S.N., Leigland, L.A., Cornea, A., Kroenke, C.D., 2012. Determination of Axonal and Dendritic Orientation Distributions Within the Developing Cerebral Cortex by Diffusion Tensor Imaging. *Medical Imaging, IEEE Transactions on* 31, 16-32.
- Jiao Y, Chen R, Ke X, Chu K, Lu Z, Herskovits EH. (2010): Predictive models of autism spectrum disorder based on brain regional cortical thickness. *NeuroImage* 50(2):589-599.
- Jones CRG, Happé F, Baird G, Simonoff E, Marsden AJS, Tregay J, Phillips RJ, Goswami U, Thomson JM, Charman T. (2009): Auditory discrimination and auditory sensory behaviours in autism spectrum disorders. *Neuropsychologia* 47(13):2850-2858.
- Jones, S.E., Buchbinder, B.R., Aharon, I., 2000. Three-dimensional mapping of cortical thickness using Laplace's equation. *Human brain mapping* 11, 12-32.
- Jou RJ, Jackowski AP, Papademetris X, Rajeevan N, Staib LH, Volkmar FR. (2011a): Diffusion Tensor Imaging in Autism Spectrum Disorders: Preliminary Evidence of Abnormal Neural Connectivity. *Australian and New Zealand Journal of Psychiatry* 45(2):153-162.

- Jou RJ, Mateljevic N, Kaiser MD, Sugrue DR, Volkmar FR, Pelphrey KA. (2011b): Structural Neural Phenotype of Autism: Preliminary Evidence from a Diffusion Tensor Imaging Study Using Tract-Based Spatial Statistics. *American Journal of Neuroradiology* 32(9):1607-1613.
- Just MA, Cherkassky VL, Keller TA, Minshew NJ. (2004): Cortical activation and synchronization during sentence comprehension in high-functioning autism: evidence of underconnectivity. *Brain* 127(8):1811-1821.
- Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. (2006): Sentence comprehension in autism: thinking in pictures with decreased functional connectivity. *Brain* 129(9):2484-2493.
- Kana RK, Keller TA, Minshew NJ, Just MA. (2007): Inhibitory Control in High-Functioning Autism: Decreased Activation and Underconnectivity in Inhibition Networks. *Biological Psychiatry* 62(3):198-206.
- Kana RK, Libero LE, Moore MS. (2011): Disrupted cortical connectivity theory as an explanatory model for autism spectrum disorders. *Physics of Life Reviews* 8(4):410-437.
- Kanabus M, Szelag E, Rojek E, Poppel E. (2002): Temporal order judgement for auditory and visual stimuli. *Acta Neurobiol. Exp.* 62:263-270.
- Kang, X., Herron, T.J., Turken, U, Woods, D.L., 2012. Diffusion properties of cortical and pericortical tissue: regional variations, reliability and methodological issues. *Magnetic Resonance Imaging* 30, 1111-1122.
- Kanner L. (1943): Autistic disturbances of affective contact. *Nervous Child* 2:217-250.
- Ke X, Hong S, Tang T, Zou B, Li H, Zhou Z, Ruan Z, Lu Z, Tao G, Liu Y. (2008): Voxel-based morphometry study on brain structure in children with high-functioning autism. *NeuroReport* 19(9):921-5.
- Ke X, Tang T, Hong S, Hang Y, Zou B, Li H, Zhou Z, Ruan Z, Lu Z, Tao G and others. (2009): White matter impairments in autism, evidence from voxel-based morphometry and diffusion tensor imaging. *Brain Research* 1265(0):171-177.
- Keary C, Minshew N, Bansal R, Goradia D, Fedorov S, Keshavan M, Hardan A. (2009): Corpus Callosum Volume and Neurocognition in Autism. *Journal of Autism and Developmental Disorders* 39(6):834-841.
- Keller TA, Kana RK, Just MA. (2007): A developmental study of the structural integrity of white matter in autism. *NeuroReport* 18(1):23-27.
- Kemper TL, Bauman ML. (1998): Neuropathology of infantile autism. *J Neuropathol Exp Neurol* 57(7):645-52.
- Kemper TL, Bauman ML. (2002): Neuropathology of infantile autism. *Mol Psychiatry* 7:S12-S13.
- Kennedy DP, Courchesne E. (2008): The intrinsic functional organization of the brain is altered in autism. *NeuroImage* 39(4):1877-1885.
- Kern JK, Trivedi MH, Garver CR, Grannemann BD, Andrews AA, Savla JS, Johnson DG, Mehta JA, Schroeder JL. (2006): The pattern of sensory processing abnormalities in autism. *Autism* 10(5):480-494.
- Kim, T.H., Zollinger, L., Shi, X.F., Rose, J., Jeong, E.K., 2009. Diffusion Tensor Imaging of Ex Vivo Cervical Spinal Cord Specimens: The Immediate and Long-Term Effects of Fixation on Diffusivity. *The Anatomical Record: Advances in Integrative Anatomy and Evolutionary Biology* 292, 234-241.
- Kirkovski M, Enticott P, Fitzgerald P. (2013): A Review of the Role of Female Gender in Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders* 43(11):2584-2603.

- Kleinhans NM, Richards T, Sterling L, Stegbauer KC, Mahurin R, Johnson LC, Greenson J, Dawson G, Aylward E. (2008): Abnormal functional connectivity in autism spectrum disorders during face processing. *Brain* 131(4):1000-1012.
- Kleinnijenhuis, M., Zerbi, V., Küsters, B., Slump, C.H., Barth, M., van Cappellen van Walsum, A.-M., 2012. Layer-specific diffusion weighted imaging in human primary visual cortex in vitro. *Cortex*.
- Klintwall L, Holm A, Eriksson M, Carlsson LH, Olsson MB, Hedvall Å, Gillberg C, Fernell E. (2011): Sensory abnormalities in autism: A brief report. *Research in Developmental Disabilities* 32(2):795-800.
- Knaus TA, Bollich AM, Corey DM, Lemen LC, Foundas AL. (2006): Variability in perisylvian brain anatomy in healthy adults. *Brain and Language* 97(2):219-232.
- Kogan MD, Blumberg SJ, Schieve LA, Boyle CA, Perrin JM, Ghandour RM, Singh GK, Strickland BB, Trevathan E, van Dyck PC. (2009): Prevalence of Parent-Reported Diagnosis of Autism Spectrum Disorder Among Children in the US, 2007. *Pediatrics* 124(5):1395-1403.
- Kolasinski, J., Stagg, C.J., Chance, S.A., DeLuca, G.C., Esiri, M.M., Chang, E.-H., Palace, J.A., McNab, J.A., Jenkinson, M., Miller, K.L., Johansen-Berg, H., 2012. A combined post-mortem magnetic resonance imaging and quantitative histological study of multiple sclerosis pathology. *Brain* 135, 2938-2951.
- Kopp S, Gillberg C. (2011): The Autism Spectrum Screening Questionnaire (ASSQ)-Revised Extended Version (ASSQ-REV): An instrument for better capturing the autism phenotype in girls? A preliminary study involving 191 clinical cases and community controls. *Research in Developmental Disabilities* 32(6):2875-2888.
- Kosaka H, Otori M, Munesue T, Ishitobi M, Matsumura Y, Takahashi T, Narita K, Murata T, Saito DN, Uchiyama H and others. (2010): Smaller insula and inferior frontal volumes in young adults with pervasive developmental disorders. *NeuroImage* 50(4):1357-1363.
- Koshino H, Carpenter PA, Minshew NJ, Cherkassky VL, Keller TA, Just MA. (2005): Functional connectivity in an fMRI working memory task in high-functioning autism. *NeuroImage* 24(3):810-821.
- Koshino H, Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. (2008): fMRI Investigation of Working Memory for Faces in Autism: Visual Coding and Underconnectivity with Frontal Areas. *Cerebral Cortex* 18(2):289-300.
- Koul O. 2006. Myelin and Autism. In: Bauman ML, Kemper TL, editors. *The Neurobiology of Autism*. Second Edition ed: The Johns Hopkins University Press.
- Kreiser N, White S. (2013): ASD in Females: Are We Overstating the Gender Difference in Diagnosis? *Clinical Child and Family Psychology Review*:1-18.
- Kubicki M, Shenton ME, Salisbury DF, Hirayasu Y, Kasai K, Kikinis R, Jolesz FA, McCarley RW. (2002): Voxel-Based Morphometric Analysis of Gray Matter in First Episode Schizophrenia. *NeuroImage* 17(4):1711-1719.
- Kumar A, Sundaram SK, Sivaswamy L, Behen ME, Makki MI, Ager J, Janisse J, Chugani HT, Chugani DC. (2010): Alterations in Frontal Lobe Tracts and Corpus Callosum in Young Children with Autism Spectrum Disorder. *Cerebral Cortex* 20(9):2103-2113.
- Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology* 1983;33:1444-1452.
- Kwakye LD, Foss-Feig JH, Cascio CJ, Stone WL, Wallace MT. (2011): Altered auditory and multisensory temporal processing in autism spectrum disorders. *Frontiers in Integrative Neuroscience* 4.

- Kwon H, Ow AW, Pedatella KE, Lotspeich LJ, Reiss AL. (2004): Voxel-based morphometry elucidates structural neuroanatomy of high-functioning autism and Asperger syndrome. *Developmental Medicine & Child Neurology* 46(11):760-764.
- Lange N, DuBray MB, Lee JE, Froimowitz MP, Froehlich A, Adluru N, Wright B, Ravichandran C, Fletcher PT, Bigler ED and others. (2010): Atypical diffusion tensor hemispheric asymmetry in autism. *Autism Res* 3(6):350-8.
- Langen M, Leemans A, Johnston P, Ecker C, Daly E, Murphy CM, dell'Acqua F, Durston S, Murphy DGM. (2012): Fronto-striatal circuitry and inhibitory control in autism: Findings from diffusion tensor imaging tractography. *Cortex* 48(2):183-193.
- Lau Y, Hinkley LN, Bukshpun P, Strominger Z, Wakahiro MJ, Baron-Cohen S, Allison C, Auyeung B, Jeremy R, Nagarajan S and others. (2013): Autism Traits in Individuals with Agenesis of the Corpus Callosum. *Journal of Autism and Developmental Disorders* 43(5):1106-1118.
- Lavie N. (2005): Distracted and confused?: Selective attention under load. *Trends in Cognitive Sciences* 9(2):75-82.
- Lawson J, Baron-Cohen S, Wheelwright S. (2004): Empathising and Systemising in Adults with and without Asperger Syndrome. *Journal of Autism and Developmental Disorders* 34(3):301-310.
- Lee JE, Bigler ED, Alexander AL, Lazar M, DuBray MB, Chung MK, Johnson M, Morgan J, Miller JN, McMahon WM and others. (2007): Diffusion tensor imaging of white matter in the superior temporal gyrus and temporal stem in autism. *Neuroscience Letters* 424(2):127-132.
- Leekam S, Nieto C, Libby S, Wing L, Gould J. (2007): Describing the Sensory Abnormalities of Children and Adults with Autism. *Journal of Autism and Developmental Disorders* 37(5):894-910.
- Leekam SR, Perner J. (1991): Does the autistic child have a metarepresentational deficit? *Cognition* 40(3):203-218.
- Leslie AM. (1987): Pretense and representation: The origins of "theory of mind". *Psychological Review* 94(4).
- Leslie AM, Thaiss L. (1992): Domain specificity in conceptual development: Neuropsychological evidence from autism. *Cognition* 43(3):225-251.
- Leuze, C.W., Dhital, B., Anwander, A., Pampel, A., Heidemann, R.M., Geyer, S., Gratz, M., Turner, R., 2011. Visualization of the Orientational Structure of the Human Stria of Gennari with High-Resolution DWI. *Proc. Intl. Soc. Mag. Reson. Med* 19.
- Leuze, C.W.U., Anwander, A., Bazin, P.-L., Dhital, B., Stüber, C., Reimann, K., Geyer, S., Turner, R., 2012. Layer-Specific Intracortical Connectivity Revealed with Diffusion MRI. *Cerebral Cortex*.
- Levitt JG, Blanton RE, Smalley S, Thompson PM, Guthrie D, McCracken JT, Sadoun T, Heinichen L, Toga AW. (2003): Cortical Sulcal Maps in Autism. *Cerebral Cortex* 13(7):728-735.
- Lewandowska M, Piatkowska-Janko E, Bogorodzki P, Wolak T, Szlag E. (2010): Changes in fMRI BOLD response to increasing and decreasing task difficulty during auditory perception of temporal order. *Neurobiology of Learning and Memory* 94(3):382-391.
- Lewine JD, Andrews R, Chez M, Patil A-A, Devinsky O, Smith M, Kanner A, Davis JT, Funke M, Jones G and others. (1999): Magnetoencephalographic Patterns of Epileptiform Activity in Children With Regressive Autism Spectrum Disorders. *Pediatrics* 104(3):405-418.
- Lewis JD, Elman JL. (2008): Growth-related neural reorganization and the autism phenotype: a test of the hypothesis that altered brain growth leads to altered connectivity. *Developmental Science* 11(1):135-155.

- Linton KF, Krcek TE, Sensui LM, Spillers JLH. (2014): Opinions of People Who Self-Identify With Autism and Asperger's on DSM-5 Criteria. *Research on Social Work Practice* 24(1):67-77.
- Liss M, Saulnier C, Fein D, Kinsbourne M. (2006): Sensory and attention abnormalities in autistic spectrum disorders. *Autism* 10(2):155-172.
- Liu, M.T., Keirstead, H.S., Lane, T.E., 2001. Neutralization of the Chemokine CXCL10 Reduces Inflammatory Cell Invasion and Demyelination and Improves Neurological Function in a Viral Model of Multiple Sclerosis. *The Journal of Immunology* 167, 4091-4097.
- Lo Y-C, Soong W-T, Gau SS-F, Wu Y-Y, Lai M-C, Yeh F-C, Chiang W-Y, Kuo L-W, Jaw F-S, Tseng W-YI. (2011): The loss of asymmetry and reduced interhemispheric connectivity in adolescents with autism: A study using diffusion spectrum imaging tractography. *Psychiatry Research: Neuroimaging* 192(1):60-66.
- Lord C, Schopler E, Revicki D. (1982): Sex differences in autism. *Journal of Autism and Developmental Disorders* 12(4):317-330.
- Lotspeich LJ, Kwon H, Schumann CM, et al. (2004): INvestigation of neuroanatomical differences between autism and aspergersyndrome. *Archives of General Psychiatry* 61(3):291-298.
- Lynch CJ, Uddin LQ, Supekar K, Khouzam A, Phillips J, Menon V. (2013): Default Mode Network in Childhood Autism: Posteromedial Cortex Heterogeneity and Relationship with Social Deficits. *Biological Psychiatry* 74(3):212-219.
- Mackenzie IS, Morant SV, Bloomfield GA, MacDonald TM, O'Riordan J. Incidence and prevalence of multiple sclerosis in the UK 1990–2010: a descriptive study in the General Practice Research Database. *Journal of Neurology, Neurosurgery & Psychiatry*. 2013.
- Mak-Fan K, Taylor M, Roberts W, Lerch J. (2012): Measures of Cortical Grey Matter Structure and Development in Children with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders* 42(3):419-427.
- Mandy W, Chilvers R, Chowdhury U, Salter G, Seigal A, Skuse D. (2012): Sex Differences in Autism Spectrum Disorder: Evidence from a Large Sample of Children and Adolescents. *Journal of Autism and Developmental Disorders* 42(7):1304-1313.
- Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y and others. (2008): Structural Variation of Chromosomes in Autism Spectrum Disorder. *The American Journal of Human Genetics* 82(2):477-488.
- Martin J, Poirier M, Bowler D. (2010): Brief Report: Impaired Temporal Reproduction Performance in Adults with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders* 40(5):640-646.
- McAlonan GM, Cheung V, Cheung C, Suckling J, Lam GY, Tai KS, Yip L, Murphy DG, Chua SE. (2005): Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. *Brain* 128(Pt 2):268-76.
- McAlonan GM, Daly E, Kumari V, Critchley HD, Amelsvoort Tv, Suckling J, Simmons A, Sigmundsson T, Greenwood K, Russell A and others. (2002): Brain anatomy and sensorimotor gating in Asperger's syndrome. *Brain* 125(7):1594-1606.
- McAlonan GM, Suckling J, Wong N, Cheung V, Lienenkaemper N, Cheung C, Chua SE. (2008): Distinct patterns of grey matter abnormality in high-functioning autism and Asperger's syndrome. *Journal of Child Psychology and Psychiatry* 49(12):1287-1295.
- McLennan J, Lord C, Schopler E. (1993): Sex differences in higher functioning people with autism. *Journal of Autism and Developmental Disorders* 23(2):217-227.

- McNab, J.A., Jbabdi, S., Deoni, S.C.L., Douaud, G., Behrens, T.E.J., Miller, K.L., 2009. High resolution diffusion-weighted imaging in fixed human brain using diffusion-weighted steady state free precession. *NeuroImage* 46, 775-785.
- McNab, J.A., Polimeni, J.R., Wang, R., Augustinack, J.C., Fujimoto, K., Stevens, A., Janssens, T., Farivar, R., Folkerth, R.D., Vanduffel, W., Wald, L.L., 2013. Surface based analysis of diffusion orientation for identifying architectonic domains in the in vivo human cortex. *NeuroImage* 69, 87-100.
- Meck WH. (2006): Neuroanatomical localization of an internal clock: A functional link between mesolimbic, nigrostriatal, and mesocortical dopaminergic systems. *Brain Research* 1109(1):93-107.
- Meck WH, Penney TB, Pouthas V. (2008): Cortico-striatal representation of time in animals and humans. *Current Opinion in Neurobiology* 18(2):145-152.
- Mengotti P, D'Agostini S, Terlevic R, De Colle C, Biasizzo E, Londero D, Ferro A, Rambaldelli G, Balestrieri M, Zanini S and others. (2011): Altered white matter integrity and development in children with autism: A combined voxel-based morphometry and diffusion imaging study. *Brain Research Bulletin* 84(2):189-195.
- Miller, K.L., McNab, J.A., Jbabdi, S., Douaud, G., 2012. Diffusion tractography of post-mortem human brains: Optimization and comparison of spin echo and steady-state free precession techniques. *NeuroImage* 59, 2284-2297.
- Miller, K.L., Stagg, C.J., Douaud, G., Jbabdi, S., Smith, S.M., Behrens, T.E.J., Jenkinson, M., Chance, S.A., Esiri, M.M., Voets, N.L., Jenkinson, N., Aziz, T.Z., Turner, M.R., Johansen-Berg, H., McNab, J.A., 2011. Diffusion imaging of whole, post-mortem human brains on a clinical MRI scanner. *NeuroImage* 57, 167-181.
- Minschew NJ, Keller TA. (2010): The nature of brain dysfunction in autism: functional brain imaging studies. *Curr Opin Neurol* 23(2):124-30.
- Monk CS, Peltier SJ, Wiggins JL, Weng S-J, Carrasco M, Risi S, Lord C. (2009): Abnormalities of intrinsic functional connectivity in autism spectrum disorders. *NeuroImage* 47(2):764-772.
- Morgan JT, Chana G, Abramson I, Semendeferi K, Courchesne E, Everall IP. (2012): Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism. *Brain Research* 1456(0):72-81.
- Morgan JT, Chana G, Pardo CA, Achim C, Semendeferi K, Buckwalter J, Courchesne E, Everall IP. (2010): Microglial Activation and Increased Microglial Density Observed in the Dorsolateral Prefrontal Cortex in Autism. *Biological Psychiatry* 68(4):368-376.
- Mori, S., Zhang, J., 2006. Principles of Diffusion Tensor Imaging and Its Applications to Basic Neuroscience Research. *Neuron* 51, 527-539.
- Moser D, Baker JM, Sanchez CE, Rorden C, Fridriksson J. (2009): Temporal Order Processing of Syllables in the Left Parietal Lobe. *The Journal of Neuroscience* 29(40):12568-12573.
- Mostafa GA, Al-Ayadhi LY. (2011): A lack of association between hyperserotonemia and the increased frequency of serum anti-myelin basic protein auto-antibodies in autistic children. *Journal of Neuroinflammation* 8.
- Mostofsky S, Goldberg M, Landa R, Denkla M. (2000): Evidence for a deficit in procedural learning in children and adolescents with autism: Implications for cerebellar contribution. *Journal of the International Neuropsychological Society* 6(07):752-759.

- Mostofsky SH, Powell SK, Simmonds DJ, Goldberg MC, Caffo B, Pekar JJ. (2009): Decreased connectivity and cerebellar activity in autism during motor task performance. *Brain* 132(9):2413-2425.
- Mottron L, Dawson M, Soulières I, Hubert B, Burack J. (2006): Enhanced Perceptual Functioning in Autism: An Update, and Eight Principles of Autistic Perception. *Journal of Autism and Developmental Disorders* 36(1):27-43.
- Mountcastle VB. (1997): The columnar organization of the neocortex. *Brain* 120(4):701-722.
- Mueller S, Keeser D, Samson AC, Kirsch V, Blautzik J, Grothe M, Erat O, Hegenloh M, Coates U, Reiser MF and others. (2013): Convergent Findings of Altered Functional and Structural Brain Connectivity in Individuals with High Functioning Autism: A Multimodal MRI Study. *PLoS ONE* 8(6):e67329.
- Mukaetova-Ladinska EB, Arnold H, Jaros E, Perry R, Perry E. (2004): Depletion of MAP2 expression and laminar cytoarchitectonic changes in dorsolateral prefrontal cortex in adult autistic individuals. *Neuropathology and Applied Neurobiology* 30(6):615-623.
- Müller R-A, Shih P, Keehn B, Deyoe JR, Leyden KM, Shukla DK. (2011): Underconnected, but How? A Survey of Functional Connectivity MRI Studies in Autism Spectrum Disorders. *Cerebral Cortex* 21(10):2233-2243.
- Munson J, Dawson G, Abbott R, et al. (2006): AMygdalar volume and behavioral development in autism. *Archives of General Psychiatry* 63(6):686-693.
- Murdoch JD, State MW. (2013): Recent developments in the genetics of autism spectrum disorders. *Current Opinion in Genetics & Development* 23(3):310-315.
- Nakano T, Ota H, Kato N, Kitazawa S. (2009): Deficit in visual temporal integration in autism spectrum disorders. *Proceedings of the Royal Society B: Biological Sciences*.
- Nickl-Jockschat T, Habel U, Maria Michel T, Manning J, Laird AR, Fox PT, Schneider F, Eickhoff SB. (2012): Brain structure anomalies in autism spectrum disorder—a meta-analysis of VBM studies using anatomic likelihood estimation. *Human Brain Mapping* 33(6):1470-1489.
- Nielsen KK, Andersen CB, Kromann-Andersen B. (1995): A Comparison Between the Effects of Paraffin and Plastic Embedding of the Normal and Obstructed Minipig Detrusor Muscle Using the Optical Dissector. *The Journal of Urology* 154(6):2170-2173.
- Nordahl CW, Dierker D, Mostafavi I, Schumann CM, Rivera SM, Amaral DG, Van Essen DC. (2007): Cortical Folding Abnormalities in Autism Revealed by Surface-Based Morphometry. *The Journal of Neuroscience* 27(43):11725-11735.
- Noriuchi M, Kikuchi Y, Yoshiura T, Kira R, Shigeto H, Hara T, Tobimatsu S, Kamio Y. (2010): Altered white matter fractional anisotropy and social impairment in children with autism spectrum disorder. *Brain Research* 1362(0):141-149.
- Nusbaum, A.O., Tang, C.Y., Buchsbaum, M.S., Wei, T.C., Atlas, S.W., 2001. Regional and Global Changes in Cerebral Diffusion with Normal Aging. *American Journal of Neuroradiology* 22, 136-142.
- Oblak AL, Gibbs TT, Blatt GJ. (2010): Decreased GABAB receptors in the cingulate cortex and fusiform gyrus in Autism. *Journal of Neurochemistry* 114(5):1414-1423.
- Oblak AL, Gibbs TT, Blatt GJ. (2011a): Reduced GABAA receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in autism. *Brain Research* 1380(0):218-228.

- Oblak AL, Rosene DL, Kemper TL, Bauman ML, Blatt GJ. (2011b): Altered posterior cingulate cortical cytoarchitecture, but normal density of neurons and interneurons in the posterior cingulate cortex and fusiform gyrus in autism. *Autism Res* 4(3):200-211.
- Ohta H, Yamada T, Watanabe H, Kanai C, Tanaka E, Ohno T, Takayama Y, Iwanami A, Kato N, Hashimoto R-i. (2012): An fMRI study of reduced perceptual load-dependent modulation of task-irrelevant activity in adults with autism spectrum conditions. *NeuroImage* 61(4):1176-1187.
- Orekhova EV, Stroganova TA, Nygren G, Tsetlin MM, Posikera IN, Gillberg C, Elam M. (2007): Excess of High Frequency Electroencephalogram Oscillations in Boys with Autism. *Biological Psychiatry* 62(9):1022-1029.
- Ozonoff S. (2012): Editorial Perspective: Autism Spectrum Disorders in DSM-5 – An historical perspective and the need for change. *Journal of Child Psychology and Psychiatry* 53(10):1092-1094.
- Palmen SJMC, van Engeland H, Hof PR, Schmitz C. (2004): Neuropathological findings in autism. *Brain* 127(12):2572-2583.
- Pardini M, Garaci FG, Bonzano L, Roccatagliata L, Palmieri MG, Pompili E, Coniglione F, Krueger F, Ludovici A, Floris R and others. (2009): White matter reduced streamline coherence in young men with autism and mental retardation. *European Journal of Neurology* 16(11):1185-1190.
- Parikshak Neelroop N, Luo R, Zhang A, Won H, Lowe Jennifer K, Chandran V, Horvath S, Geschwind Daniel H. (2013): Integrative Functional Genomic Analyses Implicate Specific Molecular Pathways and Circuits in Autism. *Cell* 155(5):1008-1021.
- Parks LK, Hill DE, Thoma RJ, Euler MJ, Lewine JD, Yeo RA. (2009): Neural correlates of communication skill and symptom severity in autism: A voxel-based morphometry study. *Research in Autism Spectrum Disorders* 3(2):444-454.
- Patenaude B, Smith SM, Kennedy DN, Jenkinson M. (2011): A Bayesian model of shape and appearance for subcortical brain segmentation. *NeuroImage* 56(3):907-922.
- Paul LK, Corsello C, Kennedy DP, Adolphs R. (2014): Agenesis of the corpus callosum and autism: a comprehensive comparison. *Brain* 137(6):1813-1829.
- Pellicano E. 2011. Psychological models of autism: an overview. In: Roth I, Rezaie P, editors. *Researching the autism spectrum*: Cambridge University Press. p 219-265.
- Penhune VB, Zatorre RJ, MacDonald JD, Evans AC. (1996): Interhemispheric Anatomical Differences in Human Primary Auditory Cortex: Probabilistic Mapping and Volume Measurement from Magnetic Resonance Scans. *Cerebral Cortex* 6(5):661-672.
- Perez Velazquez JL, Barcelo F, Hung Y, Leshchenko Y, Nenadovic V, Belkas J, Raghavan V, Brian J, Garcia Dominguez L. (2009): Decreased brain coordinated activity in autism spectrum disorders during executive tasks: Reduced long-range synchronization in the fronto-parietal networks. *International Journal of Psychophysiology* 73(3):341-349.
- Persico AM, Napolioni V. (2013): Autism genetics. *Behavioural Brain Research* 251(0):95-112.
- Peters A. 2010. The Morphology of Minicolumns. In: Blatt GJ, editor. *The Neurochemical Basis of Autism: From Molecules to Minicolumns*. New York: Springer. p 45-68.
- Peters, A., Sethares, C., Killiany, R.J., 2001. Effects of age on the thickness of myelin sheaths in monkey primary visual cortex. *The Journal of Comparative Neurology* 435, 241-248.
- Piven J, Bailey J, Ranson BJ, Arndt S. (1997): An MRI study of the corpus callosum in autism. *Am J Psychiatry* 154:1051-1056.

- Pizzarelli R, Cherubini E. (2011): Alterations of GABAergic Signaling in Autism Spectrum Disorders. *Neural Plasticity* 2011.
- Plaisted KC. 2001. Reduced generalisation in autism: an alternative to weak central coherence. In: Mahwah NJ, editor. *The Development of Autism: Perspectives from Theory and Research*: Lawrence Erlbaum Associates. p 149-169.
- Polleux F, Lauder JM. (2004): Toward a developmental neurobiology of autism. *Mental Retardation and Developmental Disabilities Research Reviews* 10(4):303-317.
- Poustka L, Jennen-Steinmetz C, Henze R, Vomstein K, Haffner J, Sieltjes B. (2012): Fronto-temporal disconnectivity and symptom severity in children with autism spectrum disorder. *The World Journal of Biological Psychiatry* 13(4):269-280.
- Powell JL, Lewis PA, Dunbar RIM, García-Fiñana M, Roberts N. (2010): Orbital prefrontal cortex volume correlates with social cognitive competence. *Neuropsychologia* 48(12):3554-3562.
- Premack D, Woodruff G. (1978): Does the chimpanzee have a theory of mind? *Behavioral and Brain Sciences* 1(4):515-526.
- Prigge MBD, Lange N, Bigler ED, Merkley TL, Neeley ES, Abildskov TJ, Froehlich AL, Nielsen JA, Cooperrider JR, Cariello AN and others. (2013): Corpus callosum area in children and adults with autism. *Research in Autism Spectrum Disorders* 7(2):221-234.
- Quester, R., Schröder, R., 1997. The shrinkage of the human brain stem during formalin fixation and embedding in paraffin. *Journal of Neuroscience Methods* 75, 81-89.
- Rademacher J, Caviness VS, Steinmetz H, Galaburda AM. (1993): Topographical Variation of the Human Primary Cortices: Implications for Neuroimaging, Brain Mapping, and Neurobiology. *Cerebral Cortex* 3(4):313-329.
- Rao SM, Mayer AR, Harrington DL. (2001): The evolution of brain activation during temporal processing. *Nat Neurosci* 4(3):317-323.
- Raymond GV, Bauman ML, Kemper TL. (1995): Hippocampus in autism: a Golgi analysis. *Acta Neuropathologica* 91(1):117-119.
- Raznahan A, Toro R, Daly E, Robertson D, Murphy C, Deeley Q, Bolton PF, Paus T, Murphy DGM. (2010): Cortical Anatomy in Autism Spectrum Disorder: An In Vivo MRI Study on the Effect of Age. *Cerebral Cortex* 20(6):1332-1340.
- Raznahan A, Wallace GL, Antezana L, Greenstein D, Lenroot R, Thurm A, Gozzi M, Spence S, Martin A, Swedo SE and others. (2013): Compared to What? Early Brain Overgrowth in Autism and the Perils of Population Norms. *Biological Psychiatry* 74(8):563-575.
- Redcay E, Courchesne E. (2005): When Is the Brain Enlarged in Autism? A Meta-Analysis of All Brain Size Reports. *Biological Psychiatry* 58(1):1-9.
- Remington A, Swettenham J, Campbell R, Coleman M. (2009): Selective Attention and Perceptual Load in Autism Spectrum Disorder. *Psychological Science* 20(11):1388-1393.
- Remington AM, Swettenham JG, Lavie N. (2012): Lightning the load: Perceptual load impairs visual detection in typical adults but not in autism. *Journal of Abnormal Psychology* 121(2):544-551.
- Ritvo E, Freeman B, Scheibel A, Duong T, Robinson H, Guthrie D, Ritvo A. (1986): Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC Autopsy Research Report. *Am J Psychiatry* 143(7):862-6.

- Riva D, Bulgheroni S, Aquino D, Di Salle F, Savoiaro M, Erbetta A. (2011): Basal Forebrain Involvement in Low-Functioning Autistic Children: A Voxel-Based Morphometry Study. *American Journal of Neuroradiology* 32(8):1430-1435.
- Roberts KL, Lau JKL, Chechlacz M, Humphreys GW. (2012): Spatial and temporal attention deficits following brain injury: A neuroanatomical decomposition of the temporal order judgement task. *Cognitive Neuropsychology* 29(4):300-324.
- Rojas DC, Peterson E, Winterrowd E, Reite ML, Rogers SJ, Tregellas JR. (2006): Regional gray matter volumetric changes in autism associated with social and repetitive behaviour symptoms. *BMC Psychiatry* 6.
- Roland PE, Geyer S, Amunts K, Schormann T, Schleicher A, Malikovic A, et al. Cytoarchitectural Maps of the Human Brain in Standard Anatomical Space. *Human Brain Mapping*. 1997;5:222-7.
- Ronald A, Happé F, Bolton P, Butcher LM, Price TS, Wheelwright S, Baron-Cohen S, Plomin R. (2006): Genetic Heterogeneity Between the Three Components of the Autism Spectrum: A Twin Study. *Journal of the American Academy of Child & Adolescent Psychiatry* 45(6):691-699.
- Rorden C. dcm2nii. <http://www.mccauslandcenter.sc.edu/micro/mricron/dcm2nii.html>
- Roxburgh RHR, Seaman SR, Masterman T, Hensiek AE, Sawcer SJ, Vukusic S, et al. Multiple Sclerosis Severity Score: Using disability and disease duration to rate disease severity. *Neurology*. 2005;64(7):1144-51.
- Rubenstein JLR, Merzenich MM. (2003): Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes, Brain and Behavior* 2(5):255-267.
- Rudick RA, Cohen JA, Weinstock-Guttman B, Kinkel RP, Ransohoff RM. Management of Multiple Sclerosis. *New England Journal of Medicine*. 1997;337(22):1604-11.
- Rutter M, Caspi A, Moffitt TE. (2003): Using sex differences in psychopathology to study causal mechanisms: unifying issues and research strategies. *Journal of Child Psychology and Psychiatry* 44(8):1092-1115.
- Sabbagh MA. (2004): Understanding orbitofrontal contributions to theory-of-mind reasoning: Implications for autism. *Brain and Cognition* 55(1):209-219
- Sahyoun CP, Belliveau JW, Mody M. (2010a): White matter integrity and pictorial reasoning in high-functioning children with autism. *Brain and Cognition* 73(3):180-188.
- Sahyoun CP, Belliveau JW, Soulières I, Schwartz S, Mody M. (2010b): Neuroimaging of the functional and structural networks underlying visuospatial vs. linguistic reasoning in high-functioning autism. *Neuropsychologia* 48(1):86-95.
- Salmond CH, Ashburner J, Connelly A, Friston KJ, Gadian DG, Vargha-Khadem F. (2005): The role of the medial temporal lobe in autistic spectrum disorders. *European Journal of Neuroscience* 22(3):764-772.
- Salmond CH, Vargha-Khadem F, Gadian DG, de Haan M, Baldeweg T. (2007): Heterogeneity in the Patterns of Neural Abnormality in Autistic Spectrum Disorders: Evidence from ERP and MRI. *Cortex* 43(6):686-699.
- Sanders Stephan J, Ercan-Sencicek AG, Hus V, Luo R, Murtha Michael T, Moreno-De-Luca D, Chu Su H, Moreau Michael P, Gupta Abha R, Thomson Susanne A and others. (2011): Multiple Recurrent De Novo CNVs, Including Duplications of the 7q11.23 Williams Syndrome Region, Are Strongly Associated with Autism. *Neuron* 70(5):863-885.
- Santos M, Uppal N, Butti C, Wicinski B, Schmeidler J, Giannakopoulos P, Heinsen H, Schmitz C, Hof PR. (2011): von Economo neurons in autism: A stereologic study of the fronto-insular cortex in children. *Brain Research* 1380(0):206-217.

- Sarachana T, Xu M, Wu R-C, Hu VW. (2011): Sex Hormones in Autism: Androgens and Estrogens Differentially and Reciprocally Regulate RORA, a Novel Candidate Gene for Autism. *PLoS ONE* 6(2):e17116.
- Sbardella E, Tona F, Petsas N, Pantano P. DTI Measurements in Multiple Sclerosis: Evaluation of Brain Damage and Clinical Implications. *Multiple Sclerosis International*. 2013;2013:11.
- Scheel C, Rotarska-Jagiela A, Schilbach L, Lehnhardt FG, Krug B, Vogeley K, Tepest R. (2011): Imaging derived cortical thickness reduction in high-functioning autism: Key regions and temporal slope. *NeuroImage* 58(2):391-400.
- Schmierer, K., Wheeler-Kingshott, C.A.M., Tozer, D.J., Boulby, P.A., Parkes, H.G., Yousry, T.A., Scaravilli, F., Barker, G.J., Tofts, P.S., Miller, D.H., 2008. Quantitative magnetic resonance of postmortem multiple sclerosis brain before and after fixation. *Magnetic Resonance in Medicine* 59, 268-277.
- Schmitz N, Rubia K, Daly E, Smith A, Williams S, Murphy DGM. (2006): Neural Correlates of Executive Function in Autistic Spectrum Disorders. *Biological Psychiatry* 59(1):7-16.
- Schumann CM, Barnes CC, Lord C, Courchesne E. (2009): Amygdala Enlargement in Toddlers with Autism Related to Severity of Social and Communication Impairments. *Biological Psychiatry* 66(10):942-949.
- Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, Lammers CR, Reiss AL, Amaral DG. (2004): The Amygdala Is Enlarged in Children But Not Adolescents with Autism; the Hippocampus Is Enlarged at All Ages. *The Journal of Neuroscience* 24(28):6392-6401.
- Schumann CM, Amaral DG. (2006): Stereological Analysis of Amygdala Neuron Number in Autism. *The Journal of Neuroscience* 26(29):7674-7679.
- Sears LL, Vest C, Mohamed S, Bailey J, Ranson BJ, Piven J. (1999): An MRI study of the basal ganglia in autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 23(4):613-624.
- Seldon HL. (1981a): Structure of human auditory cortex. I. Cytoarchitectonics and dendritic distributions. *Brain Research* 229(2):277-294.
- Seldon HL. (1981b): Structure of human auditory cortex. II. Axon distributions and morphological correlates of speech perception. *Brain Research* 229(2):295-310.
- Shah A, Frith U. (1983): An Islet of Ability in Autistic Children: A Research Note. *Journal of Child Psychology and Psychiatry* 24(4):613-620.
- Shattuck PT. (2006): The Contribution of Diagnostic Substitution to the Growing Administrative Prevalence of Autism in US Special Education. *Pediatrics* 117(4):1028-1037.
- Shukla DK, Keehn B, Lincoln AJ, Müller R-A. (2010): White Matter Compromise of Callosal and Subcortical Fiber Tracts in Children With Autism Spectrum Disorder: A Diffusion Tensor Imaging Study. *Journal of the American Academy of Child & Adolescent Psychiatry* 49(12):1269-1278.e2.
- Shukla DK, Keehn B, Müller R-A. (2011a): Tract-specific analyses of diffusion tensor imaging show widespread white matter compromise in autism spectrum disorder. *Journal of Child Psychology and Psychiatry* 52(3):286-295.
- Shukla DK, Keehn B, Smylie DM, Müller R-A. (2011b): Microstructural abnormalities of short-distance white matter tracts in autism spectrum disorder. *Neuropsychologia* 49(5):1378-1382.

- Sigalovsky, I.S., Fischl, B., Melcher, J.R., 2006. Mapping an intrinsic MR property of gray matter in auditory cortex of living humans: A possible marker for primary cortex and hemispheric differences. *NeuroImage* 32, 1524-1537.
- Simms M, Kemper T, Timbie C, Bauman M, Blatt G. (2009): The anterior cingulate cortex in autism: heterogeneity of qualitative and quantitative cytoarchitectonic features suggests possible subgroups. *Acta Neuropathologica* 118(5):673-684.
- Singh VK, Warren RP, Odell JD, Warren WL, Cole P. (1993): Antibodies to Myelin Basic Protein in Children with Autistic Behavior. *Brain, Behavior, and Immunity* 7(1):97-103.
- Sivaswamy L, Kumar A, Rajan D, Behen M, Muzik O, Chugani D, Chugani H. (2010): A Diffusion Tensor Imaging Study of the Cerebellar Pathways in Children With Autism Spectrum Disorder. *Journal of Child Neurology* 25(10):1223-1231.
- Skefos J, Cummings C, Enzer K, Holiday J, Weed K, Levy E, Yuce T, Kemper T, Bauman M. (2014): Regional Alterations in Purkinje Cell Density in Patients with Autism. *PLoS ONE* 9(2):e81255.
- Skuse DH. (2007): Rethinking the nature of genetic vulnerability to autistic spectrum disorders. *Trends in Genetics* 23(8):387-395.
- Smeeth L, Cook C, Fombonne E, Heavey L, Rodrigues LC, Smith PG, Hall AJ. (2004): Rate of first recorded diagnosis of autism and other pervasive developmental disorders in United Kingdom general practice, 1988 to 2001. *BMC Med* 2.
- Smith SM. (2002): Fast robust automated brain extraction. *Human Brain Mapping* 17(3):143-155.
- Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE and others. (2004): Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage* 23, Supplement 1(0):S208-S219.
- Solomon M, Miller M, Taylor S, Hinshaw S, Carter C. (2012): Autism Symptoms and Internalizing Psychopathology in Girls and Boys with Autism Spectrum Disorders. *Journal of Autism and Developmental Disorders* 42(1):48-59.
- Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW. (2003): Mapping cortical change across the human life span. *Nat Neurosci* 6(3):309-315.
- Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM. (2008): Towards a neuroanatomy of autism: A systematic review and meta-analysis of structural magnetic resonance imaging studies. *European Psychiatry* 23(4):289-299.
- Sundaram SK, Kumar A, Makki MI, Behen ME, Chugani HT, Chugani DC. (2008): Diffusion Tensor Imaging of Frontal Lobe in Autism Spectrum Disorder. *Cerebral Cortex* 18(11):2659-2665.
- Sunderland, S., Roche, A.F., 1958. Axon-Myelin Relationships in Peripheral Nerve Fibres. *Cells Tissues Organs* 33, 1-37.
- Supekar K, Uddin Lucina Q, Khouzam A, Phillips J, Gaillard William D, Kenworthy Lauren E, Yerys Benjamin E, Vaidya Chandan J, Menon V. (2013): Brain Hyperconnectivity in Children with Autism and its Links to Social Deficits. *Cell Reports* 5(3):738-747.
- Suren P, Stoltenberg C, Bresnahan M, Hirtz D, Lie KK, Lipkin WI, Magnus P, Reichborn-Kjennerud T, Schjolberg S, Susser E and others. (2013): Early Growth Patterns in Children with Autism. *Epidemiology* 24(5):660-670.
- Szelag E, Kowalska J, Galkowski T, Poppel E. (2004): Temporal processing deficits in high-functioning children with autism. *Br J Psychol* 95(Pt 3):269-82.

- Tannan V, Holden JK, Zhang Z, Baranek GT, Tommerdahl MA. (2008): Perceptual metrics of individuals with autism provide evidence for disinhibition. *Autism Research* 1(4):223-230.
- Taylor B, Jick H, MacLaughlin D. (2013): Prevalence and incidence rates of autism in the UK: time trend from 2004–2010 in children aged 8 years. *BMJ Open* 3(10).
- Thakkar KN, Polli FE, Joseph RM, Tuch DS, Hadjikhani N, Barton JJS, Manoach DS. (2008): Response monitoring, repetitive behaviour and anterior cingulate abnormalities in autism spectrum disorders (ASD). *Brain* 131(9):2464-2478.
- Toal F, Daly EM, Page L, Deeley Q, Hallahan B, Bloemen O, Cutter WJ, Brammer MJ, Curran S, Robertson D and others. (2010): Clinical and anatomical heterogeneity in autistic spectrum disorder: a structural MRI study. *Psychological Medicine* 40(7):1171-81.
- Tommerdahl M, Tannan V, Cascio CJ, Baranek GT, Whitsel BL. (2007): Vibrotactile adaptation fails to enhance spatial localization in adults with autism. *Brain Research* 1154(0):116-123.
- Tommerdahl M, Tannan V, Holden J, K., Baranek G, T. (2008): Absence of stimulus-driven synchronization effects on sensory perception in autism: Evidence for local underconnectivity? *Behavioral and Brain Functions* 4(19).
- Travers BG, Adluru N, Ennis C, Tromp DPM, Destiche D, Doran S, Bigler ED, Lange N, Lainhart JE, Alexander AL. (2012): Diffusion Tensor Imaging in Autism Spectrum Disorder: A Review. *Autism Res.*
- Tsai PT, Hull C, Chu Y, Greene-Colozzi E, Sadowski AR, Leech JM, Steinberg J, Crawley JN, Regehr WG, Sahin M. (2012): Autistic-like behaviour and cerebellar dysfunction in Purkinje cell Tsc1 mutant mice. *Nature* 488(7413):647-651.
- Tysza JM, Kennedy DP, Paul LK, Adolphs R. (2013): Largely Typical Patterns of Resting-State Functional Connectivity in High-Functioning Adults with Autism. *Cerebral Cortex*.
- Uddin LQ, Supekar K, Lynch CJ, et al. (2013a): SAlience network–based classification and prediction of symptom severity in children with autism. *JAMA Psychiatry* 70(8):869-879.
- Uddin LQ, Supekar K, Menon V. (2013b): Reconceptualizing functional brain connectivity in autism from a developmental perspective. *Frontiers in Human Neuroscience* 7.
- Van Essen DC, Dierker D, Snyder AZ, Raichle ME, Reiss AL, Korenberg J. (2006): Symmetry of Cortical Folding Abnormalities in Williams Syndrome Revealed by Surface-Based Analyses. *The Journal of Neuroscience* 26(20):5470-5483.
- van Kooten IAJ, Palmén SJMC, von Cappeln P, Steinbusch HWM, Korr H, Heinsen H, Hof PR, van Engeland H, Schmitz C. (2008): Neurons in the fusiform gyrus are fewer and smaller in autism. *Brain* 131(4):987-999.
- van Steinbuchel N, Wittman M, Szélag E. (1999): Temporal constraints of perceiving, generating, and integrating information: Clinical indications. *Restorative Neurology and Neuroscience* 14:167-182.
- Vattikuti S, Chow CC. (2010): A Computational Model for Cerebral Cortical Dysfunction in Autism Spectrum Disorders. *Biological Psychiatry* 67(7):672-678.
- Veluw, S., Sawyer, E., Clover, L., Cousijn, H., Jager, C., Esiri, M., Chance, S., 2012. Prefrontal cortex cytoarchitecture in normal aging and Alzheimer's disease: a relationship with IQ. *Brain Structure and Function* 217, 797-808.
- Vidal CN, Nicolson R, DeVito TJ, Hayashi KM, Geaga JA, Drost DJ, Williamson PC, Rajakumar N, Sui Y, Dutton RA and others. (2006): Mapping Corpus Callosum Deficits in Autism: An Index of Aberrant Cortical Connectivity. *Biological Psychiatry* 60(3):218-225.

- Villalobos ME, Mizuno A, Dahl BC, Kemmotsu N, Müller R-A. (2005): Reduced functional connectivity between V1 and inferior frontal cortex associated with visuomotor performance in autism. *NeuroImage* 25(3):916-925.
- Vinters HV, Kleinschmidt-DeMasters BK. 2008. General pathology of the central nervous system. In: Love S, Louis DN, Ellison DW, editors. *Greenfield's Neuropathology*. London: Hodder Arnold. p 1-62.
- Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Mill J, Cantor RM, Blencowe BJ, Geschwind DH. (2011): Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474(7351):380-384.
- Volkmar F, Szatmari P, Sparrow S. (1993): Sex differences in pervasive developmental disorders. *Journal of Autism and Developmental Disorders* 23(4):579-591.
- Volkmar FR, Reichow B. (2013): Autism in DSM-5: progress and challenges. *Molecular Autism* 4.
- Völlm BA, Taylor ANW, Richardson P, Corcoran R, Stirling J, McKie S, Deakin JFW, Elliott R. (2006): Neuronal correlates of theory of mind and empathy: A functional magnetic resonance imaging study in a nonverbal task. *NeuroImage* 29(1):90-98.
- von dem Hagen EAH, Stoyanova RS, Baron-Cohen S, Calder AJ. (2013): Reduced functional connectivity within and between 'social' resting state networks in autism spectrum conditions. *Social Cognitive and Affective Neuroscience* 8(6):694-701.
- Von Economo, C., Koskinas, G.N., 1925. *Die Cytoarchitektonik der Hirnrinde des Erwachsenen Menschen*. Springer, Berlin (Germany).
- Vrenken, H., Pouwels, P.J.W., Geurts, J.J.G., Knol, D.L., Polman, C.H., Barkhof, F., Castelijns, J.A., 2006. Altered diffusion tensor in multiple sclerosis normal-appearing brain tissue: Cortical diffusion changes seem related to clinical deterioration. *Journal of Magnetic Resonance Imaging* 23, 628-636.
- Waiter GD, Williams JHG, Murray AD, Gilchrist A, Perrett DI, Whiten A. (2004): A voxel-based investigation of brain structure in male adolescents with autistic spectrum disorder. *NeuroImage* 22(2):619-625.
- Wallace GL, Dankner N, Kenworthy L, Giedd JN, Martin A. (2010): Age-related temporal and parietal cortical thinning in autism spectrum disorders. *Brain* 133(12):3745-3754.
- Washington SD, Gordon EM, Brar J, Warburton S, Sawyer AT, Wolfe A, Mease-Ference ER, Girton L, Hailu A, Mbwana J and others. (2013): Dysmaturation of the default mode network in autism. *Human Brain Mapping*:n/a-n/a.
- Wass S. (2011): Distortions and disconnections: Disrupted brain connectivity in autism. *Brain and Cognition* 75(1):18-28.
- Wazana A, Bresnahan M, Kline J. (2007): The Autism Epidemic: Fact or Artifact? *Journal of the American Academy of Child & Adolescent Psychiatry* 46(6):721-730.
- Wegiel J, Kuchna I, Nowicki K, Imaki H, Wegiel J, Marchi E, Ma S, Chauhan A, Chauhan V, Bobrowicz T and others. (2010): The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. *Acta Neuropathologica* 119(6):755-770.
- Weidenheim KM, Goodman L, Dickson DW, Gillberg C, Råstam M, Rapin I. (2001): Etiology and Pathophysiology of Autistic Behavior: Clues From Two Cases With an Unusual Variant of Neuroaxonal Dystrophy. *Journal of Child Neurology* 16(11):809-819.

- Weinstein M, Ben-Sira L, Levy Y, Zachor DA, Itzhak EB, Artzi M, Tarrasch R, Eksteine PM, Hendler T, Bashat DB. (2011): Abnormal white matter integrity in young children with autism. *Human Brain Mapping* 32(4):534-543.
- Welchew DE, Ashwin C, Berkouk K, Salvador R, Suckling J, Baron-Cohen S, Bullmore E. (2005): Functional disconnectivity of the medial temporal lobe in Asperger's syndrome. *Biological Psychiatry* 57(9):991-998.
- Welsh JP, Ahn ES, Placantonakis DG. (2005): Is autism due to brain desynchronization? *International Journal of Developmental Neuroscience* 23(2-3):253-263.
- Weng S-J, Wiggins JL, Peltier SJ, Carrasco M, Risi S, Lord C, Monk CS. (2010): Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. *Brain Research* 1313(0):202-214.
- Werling DM, Geschwind DH. (2013): Sex differences in autism spectrum disorders. *Curr Opin Neurol* 26(2):146-53.
- Williams J, Allison C, Scott F, Bolton P, Baron-Cohen S, Matthews F, Brayne C. (2008): The Childhood Autism Spectrum Test (CAST): Sex Differences. *Journal of Autism and Developmental Disorders* 38(9):1731-1739.
- Williams, P.L., Wendell-Smith, C.P., 1971. Some additional parametric variations between peripheral nerve fibre populations. *J Anat* 109, 505-526.
- Williams RS, Hauser SL, Purpura DP, DeLong G, Swisher CN. (1980): Autism and mental retardation: Neuropathologic studies performed in four retarded persons with autistic behavior. *Archives of Neurology* 37(12):749-753.
- Willsey AJ, Sanders Stephan J, Li M, Dong S, Tebbenkamp Andrew T, Muhle Rebecca A, Reilly Steven K, Lin L, Fertuzinhos S, Miller Jeremy A and others. (2013): Coexpression Networks Implicate Human Midfetal Deep Cortical Projection Neurons in the Pathogenesis of Autism. *Cell* 155(5):997-1007.
- Wilson TW, Rojas DC, Reite ML, Teale PD, Rogers SJ. (2007): Children and Adolescents with Autism Exhibit Reduced MEG Steady-State Gamma Responses. *Biological Psychiatry* 62(3):192-197.
- Wimmer H, Perner J. (1983): Beliefs about beliefs: Representation and constraining function of wrong beliefs in young children's understanding of deception. *Cognition* 13(1):103-128.
- Wolff JJ, Gu H, Gerig G, Elison JT, Styner M, Gouttard S, Botteron KN, Dager SR, Dawson G, Estes AM and others. (2012): Differences in White Matter Fibre Tract Development Present From 6 to 24 Months in Infants With Autism. *American Journal of Psychiatry* 169(6).
- Woo S-H, Kim K-H, Lee K-M. (2009): The role of the right posterior parietal cortex in temporal order judgment. *Brain and Cognition* 69(2):337-343.
- Woolrich MW, Jbabdi S, Patenaude B, Chappell M, Makni S, Behrens T, Beckmann C, Jenkinson M, Smith SM. (2009): Bayesian analysis of neuroimaging data in FSL. *NeuroImage* 45(1, Supplement 1):S173-S186.
- Yeargin-Allsopp M. (2008): The prevalence and characteristics of autism spectrum disorders in the ALSPAC cohort. *Developmental Medicine & Child Neurology* 50(9):646-646.
- Yu KK, Cheung C, Chua SE, McAlonan GM. (2011): Can Asperger syndrome be distinguished from autism? An anatomical likelihood meta-analysis of MRI studies. *J Psychiatry Neurosci* 36(6):412-21.

- Zhang Y, Brady M, Smith S. (2001): Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *Medical Imaging, IEEE Transactions on* 20(1):45-57.
- Ziats M, Rennert O. (2013): Aberrant Expression of Long Noncoding RNAs in Autistic Brain. *Journal of Molecular Neuroscience* 49(3):589-593.
- Zielinski BA, Prigge MBD, Nielsen JA, Froehlich AL, Abildskov TJ, Anderson JS, Fletcher PT, Zygmunt KM, Travers BG, Lange N and others. (2014): Longitudinal changes in cortical thickness in autism and typical development. *Brain* 137(6):1799-1812.
- Zikopoulos B, Barbas H. (2010): Changes in Prefrontal Axons May Disrupt the Network in Autism. *The Journal of Neuroscience* 30(44):14595-14609.
- Zikopoulos B, Barbas H. (2013): Altered neural connectivity in excitatory and inhibitory cortical circuits in autism. *Frontiers in Human Neuroscience*.

Appendix – Histology Staining Methods

Cresyl Violet

- i) Filter Cresyl Violet solution
- ii) Wash sections in distilled water
- iii) Place sections in 0.1% Cresyl Violet for 60 seconds
- iv) Wash sections in 70% alcohol
- v) Transfer sections to 90% alcohol (60 dips)
- vi) Transfer sections to 100% alcohol (10 dips)
- vii) Transfer sections to histoclear (5 mins)
- viii) Coverslip sections using DPX

0.1% Cresyl Violet Solution:

| | |
|--------|-----------------|
| 0.5g | Cresyl Violet |
| 500 ml | Distilled Water |
| 4ml | 10% Acetic Acid |

Sudan Black

- i) Filter Sudan Black solution twice
- ii) Wash sections in distilled water
- iii) Place sections in Sudan Black solution (15 mins)
- iv) Refresh Sudan Black solution (15 mins)
- v) Transfer sections to 70% alcohol (30 dips)
- vi) Transfer sections to distilled water
- vii) Coverslip sections with glycerol aqueous coverslip medium

Sudan Black Solution:

| | |
|--------|-------------|
| 1.25g | Sudan Black |
| 500 ml | 70% Ethanol |

Glycerol Aqueous Coverslip Medium:

| | |
|-------|-----------------|
| 5g | Gelatin |
| 50 ml | Distilled Water |
| 50 ml | Glycerol |