Structural brain abnormalities in autism

Saskia Palmen

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Voor
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en
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Chapter 1

Autism

Autism is a neurodevelopmental disorder of unknown origin defined by the presence of marked social deficits, specific language abnormalities, and stereotyped, repetitive behaviors [American Psychiatric Association 1994] (table 1-1). Behavioral abnormalities are typically observed before the age of 30 months is reached and persist throughout life, although symptoms generally become less severe with age [Korkmaz 2000;Rumsey et al. 1985a]. Currently, autism is considered to be the most strongly genetically influenced childhood psychiatric disorder [Andres 2002;Bailey et al. 1995;Lauritsen and Ewald 2001;Trottier et al. 1999;Turner et al. 2000], with an estimated heritability of more than 90% [Bailey et al. 1996]. In a recent review, the median prevalence estimate of autism was 1.1/1000 [Fombonne 2003], with boys four times more affected than girls [Ciaranello and Ciaranello 1995;McLennan et al. 1993;Yeargin-Allsopp et al. 2003]. A positive correlation was found between prevalence rate and year of publication, pointing towards an increase in prevalence of autism in the last 15 years, which is most likely due to changes in case definition and improved awareness, rather than a true increase in incidence [Fombonne 2003]. In approximately 70%, autism is accompanied by mental retardation and in about 60% by epilepsy [Bailey et al. 1996;Yeargin-Allsopp et al. 2003]. In 6% of cases, causal neurological diseases such as tuberous sclerosis, neurofibromatosis, fragile X syndrome and phenylketonuria have been found [Fombonne 1999]. Thus, in the majority of autism cases, the exact etiology still remains to be determined.

Table 1-1: Diagnostic criteria of autistic disorder, according to DSM-IV

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

1. Qualitative impairment in social interaction, as manifested by at least two of the following:
   - marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate 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

2. Qualitative impairment in communication as manifested by at least one of the following:
   - 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

3. Restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following:
   - encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
   - apparently inflexible adherence to specific, nonfunctional, routines or rituals
   - stereotyped and repetitive motor mannerisms (e.g. hand or finger flapping or twisting, or complex whole body movements)
   - persistent preoccupation with parts of objects

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

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

After setting out the scope of this thesis, we will use the remainder of this introduction for discussing findings from the various fields of autism research, i.e. genetics, neuropsychology and its functional correlate, structural brain research, and neurochemistry, as all these fields of research, especially when combined, will add to resolving the underlying causes of autism. The literature, most relevant to this thesis (structural MRI and neuropathology), is reviewed in separate chapters, i.e. chapter 2 and chapter 7 respectively. The literature of the remaining fields of research, however, should by no means be viewed as reviews, as the scope of these fields is much larger than that which is discussed here.

The scope of the present thesis

To date, an accumulating body of evidence suggests that head and brain size in autism is, on average, increased. Kanner, the first to report on autism, noticed the presence of enlarged heads in some children with autism [Kanner 1943]. Following this first report, numerous other studies found macrocephaly in approximately 20% of subjects with autism [Aylward et al. 2002;Bailey et al. 1993;Davidovitch et al. 1996;Fidler et al. 2000;Fombonne 2000;Lainhart et al. 1997;Miles et al. 2000;Naqvi et al. 2000;Stevenson et al. 1997;van Karnebeek et al. 2002;Woodhouse et al. 1996]. Consistent with these clinical findings, neuropathological studies have reported increased brain weight in autistic individuals, especially children [Bailey et al. 1998;Casanova et al. 2002;Courchesne et al. 1999;Kemper and Bauman 1998]. In addition, neuroimaging studies reported increased brain volume in autistic children [Aylward et al. 2002;Carper et al. 2002;Courchesne et al. 2001;Filipek et al. 1992;Herbert et al. 2003;Sparks et al. 2002]. However, in adolescents and adults results were inconsistent with reports of either increased brain volume [Hardan et al. 2001a;Piven et al. 1995;Piven et al. 1996], or no difference in brain volume [Aylward et al. 1999;Aylward et al. 2002;Carper et al. 2002;Courchesne et al. 2001;Haznedar et al. 2000;Rojas et al. 2002;Townsend et al. 2001] in autistic subjects compared to controls. Thus, the question seems to be whether brain enlargement in autism is a phenomenon restricted to early childhood or whether it remains present in older children, adolescents, and even adults. Two related, and still unanswered, questions are whether this enlargement would be confined to the gray and/or the white matter and whether the enlargement would be global or more prominent in specific brain regions, all question that can be answered with structural MRI research.

More basically, the underlying biological mechanisms of brain enlargement remain to be determined and could involve increased neurogenesis, increased gliogenesis, increased synaptogenesis, disturbed migration of neurons, decreased apoptosis, decreased pruning, or complex combinations of these events. While combining neuropathological and neuroimaging research, the goal of this thesis is to explore and to add to the existing knowledge of structural brain abnormalities in autism.

Using Magnetic Resonance Imaging (MRI), it is possible to examine the brain in vivo. Although MRI has the big advantage of the possibility of (repeatedly) scanning of (young) children, individual cells and cell layers cannot be visualized, thus precluding an assessment of the underlying cause of differences in brain shape and volume. On the other hand, modern neuropathological techniques, such as design-based, observer-independent stereology, do offer this possibility. In this thesis, both structural MRI and –for the first time in autism research- stereology techniques are used to better define the anatomical abnor-

malities found in autism. Cautious statements about the possible underlying fundamental disturbances will be made, based on the findings in the present thesis. Finally, future directions of research will be touched upon.

The genetics of autism

Overwhelming evidence exists for a large genetic component in autism (for example see [Andres 2002;Bailey et al. 1995;Lauritsen and Ewald 2001;Trottier et al. 1999;Turner et al. 2000]). Twin studies show a 60-90% concordance rate for autism in monozygotic twins, contrary to concordance rates between 0% and 20 % in dizygotic twins [Bailey et al. 1995;Ritvo et al. 1985;Steffenburg et al. 1989]. The sibling-recurrence-risk ratio, estimated from epidemiological studies, ranges from 50 to 150 [Folstein and Piven 1991;Jorde et al. 1991;Smalley et al. 1988]. This pattern of sharply increasing risk for first-degree relatives and monozygotic twins relative to the population prevalence (~1.1/1000 [Fombonne 2003]) does not fit a simple dominant or recessive model, but rather indicates the involvement of multiple genes interacting with one another to lead to disease susceptibility.

There are three main approaches to identifying chromosomal regions likely to contain relevant genes (for reviews, see [Lamb et al. 2000;Muhle et al. 2004;Veenstra-VanderWeele and Cook 2004]): 1) whole-genome screens, searching for linkage of autism to shared genetic markers in populations of multiple affected families; 2) cytogenetic studies, pointing to relevant inherited or de novo chromosomal abnormalities in affected individuals and their families; and 3) evaluation of candidate genes known to affect brain development in these significantly linked regions, or, alternatively, linkage of candidate genes selected a priori because of their presumptive contribution to the pathogenesis of autism.

The results from whole-genome screens indicate that, most likely, 10 or more genes interact to cause autism [Pickles et al. 1995;Risch et al. 1999;Shao et al. 2002], with evidence of involvement of chromosome 1p [Auranen et al. 2000;Risch et al. 1999], 2q [Buxbaum et al. 2001;International Molecular Genetic Study of Autism Consortium (IMGSAC) 2001a;Philippe et al. 1999;Shao et al. 2002], 3q [Auranen et al. 2002;Shao et al. 2002], 4p and 4q [International Molecular Genetic Study of Autism Consortium (IMGSAC) 1998;Philippe et al. 1999], 5p [Liu et al. 2001;Philippe et al. 1999], 6q [Philippe et al. 1999], 7q(31-33) [Alarcon et al. 2002;Ashley-Koch et al. 1999;Barrett et al. 1999;International Molecular Genetic Study of Autism Consortium (IMGSAC) 1998;International Molecular Genetic Study of Autism Consortium (IMGSAC) 2001b;International Molecular Genetic Study of Autism Consortium (IMGSAC)

2001a;Philippe et al. 1999;Risch et al. 1999;Shao et al. 2002], 10q [International Molecular Genetic Study of Autism Consortium (IMGSAC) 1998;Philippe et al. 1999], 13q [Barrett et al. 1999;Risch et al. 1999], 15q(11-13) [Philippe et al. 1999;Shao et al. 2003;Shao et al. 2002], 16p [International Molecular Genetic Study of Autism Consortium (IMGSAC) 1998;International Molecular Genetic Study of Autism Consortium (IMGSAC) 2001a;Liu et al. 2001;Philippe et al. 1999], 17q [International Molecular Genetic Study of Autism Consortium (IMGSAC) 2001a], 18q [Philippe et al. 1999], 19p [International Molecular Genetic Study of Autism Consortium (IMGSAC) 1998;Liu et al. 2001;Philippe et al. 1999;Shao et al. 2002], 22q [International Molecular Genetic Study of Autism Consortium (IMGSAC) 1998], and Xp [Liu et al. 2001;Philippe et al. 1999;Shao et al. 2002].

Cytogenetic abnormalities have been found on almost every chromosome [Gillberg 1998;Veenstra-VanderWeele and Cook 2004]. However, only for a limited number of regions on the human genome, multiple cases have been reported. These regions include chromosome 1p22.2; terminal part of chromosome 2q; distal short arm of chromosome 5; widespread regions on chromosome 7 –among which the reelin protein (RELN)- [Ashley-Koch et al. 1999;Gillberg 1998;Scherer et al. 2003;Yan et al. 2000]; chromosome 15q11-q14 -containing the GABA receptor gene cluster- [Cook et al. 1998;Gillberg 1998;Martin et al. 2000;Menold et al. 2001;Rineer et al. 1998;Schroer et al. 1998]; and chromosome 22q11.2 and q13 [Vorstman et al. 2004].

As almost all neurochemical systems –serotonergic [Anderson 2002;Chugani 2002;McDougle and Posey 2002], dopaminergic [Ernst et al. 1997;McDougle and Posey 2002], cholinergic [Lee et al. 2002;Perry et al. 2001], GABAergic [Cook et al. 1998;Hussman 2001;Schroer et al. 1998], glutamatergic [Carlsson 1998;Nilsson et al. 2001], and endogenous opioid [Panksepp 1979;Sahley and Panksepp 1987]- are suggested to play a role in the etiology of autism, genes that code for the receptors or neurotransmitters of these systems are candidate genes. Unfortunately, results from these hypothesis-driven studies are equivocal [Cook, Jr. et al. 1997;Klauck et al. 1997;Philippe et al. 2002;Risch et al. 1999;Robinson et al. 2001;Tordjman et al. 2001;Yirmiya et al. 2001].

Thus, although the evidence for genetic factors in autism is overwhelming, it remains to be elucidated which genes are responsible. Furthermore, one should bear in mind that environmental factors, such as exposure to drugs (thalidomide [Stromland et al. 1994], valproate acid [Ingram et al. 2000;Williams et al. 2001], and ethanol [Nanson 1992]) and pre- and perinatal risk factors, such as previous abortion, uterine bleeding, maternal or fetal infection, prolonged labor, fetal distress, and low birth weight [Burd et al. 1999;Deykin and MacMahon 1980;Finegan and Quarrington 1979;Gillberg and Gillberg 1983;Glasson et al. 2004;Juul-Dam et al. 2001;Mason-Brothers et al. 1990] also contribute to the etiology of autism.

The neuropsychology of autism and its functional correlate

Since the early 1990s, three somewhat overlapping psychological models of autism have emerged (for review, see [Deuel 2002]): 1) the theory of mind (ToM) hypothesis, suggesting that individuals with autism have difficulties in making attributions of mental states to others, leading to an inability to predict the behaviors of others, with consequent actions that are socially aberrant [Baron-Cohen et al. 1997;Happé 1994;Heavey et al. 2000;Klin 2000]; 2) the central coherence hypothesis [Happé and Frith 1996], manifesting itself as the tendency of individuals with autism to process stimuli in a fragmented fashion, focusing on details, rather than combining diverse and detailed internal information into relevant higher-order concepts that guide behavior over the long term [Briskman et al. 2001;Jolliffe and Baron-Cohen 2000]; and 3) the executive dysfunction hypothesis [Pennington and Ozonoff 1996], stating that learning in autism is characterized by perseveration and poor self-regulation, including difficulties with change, reduced forward planning, and ineffective problem-solving skills, leading to an inability to shift (promote or inhibit) thought or motor output strategies toward a current performance demand [Hughes et al. 1994;Ozonoff et al. 1991].

As the functional substrate of these three prevailing theories can best be tested with functional neuroimaging studies (using social recognition tasks, embedded figure tasks, and attention (shifting) tasks), we will discuss them in that context.

Functional brain imaging studies provide information about brain activity, either in rest or in response to specific tasks. In the early eighties, the first functional brain imaging studies in autism appeared, producing quite contradictory results. Positron emission tomography (PET) studies look at brain glucose metabolism, as an indirect measure of neuronal activity. Single photon emission computed tomography (SPECT) measures regional cerebral blood flow (rCBF). However, since both techniques make use of radioactive tracers, the ethical constraints for control groups are very strict. More recently, functional MRI (fMRI) was introduced into the field of autism research. This technique provides information about localized changes in blood flow –an indirect measure of underlying neural activity- by employing an endogenous contrast property of the brain, blood oxygen level dependent contrast (BOLD) and can be performed without tracer substances, which makes it very well suited for (repeatedly) scanning of young children. In the last decade, the functional imaging techniques have improved dramatically, resulting in more unequivocal results, although the variety in tasks performed during functional imaging studies makes it difficult to compare functional studies one to another. In the

next section, the most important findings in the field of functional neuroimaging in autism will be discussed (for extensive reviews, see [Boddaert and Zilbovicius 2002;Cody et al. 2002]).

The majority of functional imaging studies, performed at rest, reported decreased CBF or glucose utilization [Asano et al. 2001;Chiron et al. 1995;George et al. 1992;Kaya et al. 2002;McKelvey et al. 1995;Mountz et al. 1995;Ohnishi et al. 2000;Ryu et al. 1999;Sherman et al. 1984;Wilcox et al. 2002;Zilbovicius et al. 2000;Zilbovicius et al. 1995], although reports of increased CBF or glucose metabolism [Asano et al. 2001;Horwitz et al. 1988;Rumsey et al. 1985b] and studies, reporting no differences between autistic subjects and controls [De Volder et al. 1987;Herold et al. 1988;Schifter et al. 1994;Zilbovicius et al. 1992] have been published as well. These contradictory findings can be partly ascribed to 1) the heterogeneity of the disorder itself; 2) the lack of matching between and within studies; and 3) methodological differences, such as scanning environment (in light or in dark environment), (no) sedation, and the use of more sophisticated techniques in recent years. Of more importance, however, is the fact that scanning 'at rest' by no means guarantees that there is no activity in the brains of the tested subjects (e.g. tell somebody not to think of a white bear, and he will immediately start to think about one).

Apart from functional imaging studies, performed during rest, several tasks, tapping on different domains of impairment in autism –including the three neuropsychological theories of autism-, have been used to investigate possible abnormalities in brain activation in autism.

First of all, social cognition -the most severely impaired domain of autism- has been tested. The emotional and social brain network consists of 10 regions, among which the fusiform gyrus -important for face discrimination or face identification-, the superior temporal sulcus (STS) -sensitive to gaze direction of another person's eyes-, and the amygdala -critical for emotional processing (especially left), modulation of memory and fear conditioning. The other seven regions are located in the prefrontal cortex and the anterior cingulate cortex (for extensive descriptions see [Grady and Keightley 2002]). To date, 13 studies investigated social cognition in autism, one testing "Theory of Mind" abilities [Happé et al. 1996], one investigating social judgement [Oktem et al. 2001], and eleven measuring facial recognition abilities [Baron-Cohen et al. 1999;Critchley et al. 2000;Hadjikhani et al. 2004;Hall et al. 2003;Hubl et al. 2003;Ogai et al. 2003;Pierce et al. 2001;Pierce et al. 2004;Piggot et al. 2004;Schultz et al. 2000;Wang et al. 2004]. Overall, there seems to be agreement on the fact that brain activation in social cognition is abnormal in autism. Results are unequivocal regarding temporal lobe abnormalities, especially decreased activation in the fusiform gyrus and the amygdala during facial expression tasks, although one study found normal fusiform activity when

autistic adults looked at familiar faces [Pierce et al. 2004]. In addition, the frontal lobes are less activated in autistic subjects during social recognition. It was suggested that autistic subjects activate aberrant and individual-specific regions, implying the use of alternative cortical regions as a sort of compensation strategy [Baron-Cohen et al. 1999; Hall et al. 2003;Pierce et al. 2001;Schultz et al. 2000].

Although language is one of the key domains, known to be impaired in autism, to date only four studies investigated language impairments in autism [Boddaert et al. 2003;Just et al. 2004;Müller et al. 1998;Müller et al. 1999]. Overall, results were consistent with reversed hemispheric dominance for language in autism. Most recently increased activation in Wernicke's (left latero-superior temporal) area and less activation in Broca's (left inferior frontal gyrus) area were found in autistic subjects during sentence comprehension [Just et al. 2004]. In addition, the functional connectivity, i.e. the degree of synchronization or correlation of the time series of the activation, between the various participating cortical areas was consistently lower for the autistic than the control participants [Just et al. 2004].

Impairments in sustained attention have been suggested previously in autism [Courchesne et al. 1994;Rapin and Katzman 1998;Waterhouse et al. 1996]. Several functional imaging studies used a continuous performance test (CPT) paradigm to test attention in autism. Overall, decreased activation, especially in the cerebellum, was reported in autism during the CPT, with a reversal of the normal right > left asymmetry [Allen and Courchesne 2003;Buchsbaum et al. 1992;Siegel, Jr. et al. 1992], or aberrant activation of occipital and striate cortex [Belmonte and Yurgelun-Todd 2003]. However, reports of absence of abnormalities in activation during attention tasks were also reported [Heh et al. 1989;Siegel, Jr. et al. 1995].

Apart from impairments in several domains of social and cognitive functioning, subjects with autism have been reported to demonstrate superiority over normal controls on tests of local processing and visual search, exemplified by the Embedded Figure Task (EFT) [Jolliffe and Baron-Cohen 1997;Shah and Frith 1983]. Using this EFT, memory function was tested in three studies [Haznedar et al. 2000;Luna et al. 2002;Ring et al. 1999]. These functional imaging studies of working memory consistently showed decreased activation in regions normally used for working memory –dorsolateral prefrontal cortex and cingulate cortex-, while performance was comparable between the autism groups and the control groups. In addition, activation of aberrant, compensational regions, especially occipitotemporal, was reported. Recently, subjects with autism showed dysfunction in some –predominantly cingulate- key brain regions subserving verbal memory performance, whereas additional, compensatory aberrant regions, in parietal and occipital cortex, were recruited [Hazlett et al. 2004].

In conclusion, some neurofunctional results appear to be quite consistent: 1) during rest, most studies reported decreased activation in autism, predominantly in the frontal and temporal regions; 2) during social cognition tasks, autistic subjects showed decreased activation of the fusiform gyrus, amygdala, and frontal regions, with individual-specific aberrant regions of activation; 3) during language tasks, absence of the left hemisphere dominance for language was found in autistic subjects; and 4) memory tasks revealed decreased frontal and cingulate activation.

The anatomical abnormalities of autism

Starting at the cellular level, neuropathological studies have come up with some consistent results, although the majority of the neuropathological data remains equivocal, largely due to the lack of consistent design in histopathological quantitative studies. For an extensive review of the neuropathological data, see Chapter 7. In short, when considering the available data, a number of conclusions can be drawn. First, a decrease in the number of Purkinje cells throughout the cerebellar hemispheres without significant gliosis and features of cortical dysgenesis have been consistently found by different research groups. Second, although not replicated by independent research groups, increased cell packing density of smaller neurons in the limbic system and age-related abnormalities in the cerebellar nuclei and the inferior olive have been reported in the majority of the studied cases. Finally, both the cholinergic and the GABAergic system seem to be impaired in autism.

While research on the neuropathology of autism has been hampered by the lack of availability of large sample sizes and closely matched control groups, structural MRI, on the other hand, is uniquely suited to (repeatedly) scan the brains of large groups of (young) patients and closely matched controls in vivo and map neuroanatomic abnormalities. Unfortunately, to date, structural MRI findings cannot be directly correlated to the neuropathological findings in autism, although the repeatedly reported increased brain volume detected with MRI [Aylward et al. 2002;Courchesne et al. 2001;Filipek et al. 1992;Hardan et al. 2001a;Piven et al. 1995;Sparks et al. 2002] seems consistent with the frequent observation of an increased brain weight in autism [Bailey et al. 1998;Casanova et al. 2002;Courchesne et al. 1999;Kemper and Bauman 1998]. The available literature on structural MRI abnormalities in autism is extensively reviewed in the next chapter, but in short: one of the most reliable findings about brains in autism is their increased cerebral volume, at least in children [Frith 2002]; cerebellar volume is likely to be increased as well; in contrast, the (posterior) midsagittal corpus callosum is likely to be smaller; and some consensus seems

to exist towards enlargement of the amygdala. However, three important questions –which will be dealt with in chapters 3-5- are still unanswered: 1) is brain enlargement in autism a phenomenon restricted to early childhood or is it still present in older children, adolescents, and even adults; 2) is enlargement confined to the gray and/or the white matter; and 3) is the enlargement global or more prominent in specific brain regions.

The neuropharmacology of autism

Although several neurotransmitter systems are suggested to play a role in the etiology of autism, drugs that have consistent, primary effects on the core social and communication disability of autism have not yet been developed. Up till now the pharmacotherapy of autism involves the treatment of target symptoms, such as hyperactivity, inattention, interfering repetitive thoughts and behaviors, aggression, and anxiety [McDougle and Posey 2002].

Serotonergic system: Serotonin receptors affect various cognitive functions such as memory, executive functioning, and language, all known to be impaired in autism [Abu-Akel 2003]. Indeed, studies have shown a dysfunction in the serotonergic system [Cook 1990;Cook and Leventhal 1996]. The most consistent finding has been increased whole-blood-serotonin in autism [Anderson et al. 1987;Cook and Leventhal 1996;Mulder et al. 2004;Singh et al. 1997], which is suggested to be the result of a defect in serotonin biochemistry of the dentatothalamocortical pathway [Chugani et al. 1997]. Selective serotonin re-uptake inhibitors (SSRIs), such as fluoxetine, cause decrease in repetitive thoughts, aggression, and anxiety [Deuel 2002;Eigsti and Shapiro 2003;Posey and McDougle 2000].

Dopaminergic system: Dopamine is involved in motor activity, attention skills, modulating social behavior and regulating emotional responses, all aspects, known to be impaired in autism [Nieminen-von Wendt et al. 2004]. Indeed, low medial prefrontal dopaminergic activity was reported in autistic children [Ernst et al. 1997]. Atypical antipsychotics, such as risperidone, block dopamine -and serotonin- receptors and cause decrease in hyperactivity, aggression, repetitive behavior [Barnard et al. 2002;Eigsti and Shapiro 2003].

Glutamatergic system: Autism was suggested to be a hypoglutamatergic disorder as symptoms evoked by glutamate antagonists –blocking the N-methyl-D-aspartate (NMDA) receptor- are very similar to those of autism, especially perceptual disturbances and perseverant focus on trivial details at the cost of a general view [Carlsson 1998]. Recently, the involvement of glutamate in autism was confirmed in an association study; a polymorphism in the glutamate receptor on chromosome 6 was found more often in autistic subjects (8%)

compared to controls (4%) [Jamain et al. 2002]. Unfortunately, treatment with glutamate agonists, although the most obvious thing to do, is a hazardous task, since excessive glutamate receptor stimulation results in neurotoxicity and convulsions.

GABAergic system: The GABAergic system is the largest inhibitory system in the cerebral cortex [Rubenstein and Merzenich 2003]. It is suggested that a decrease in the availability of these inhibitory receptors can increase receptor activity. As a consequence, the threshold for development of seizures, a frequent comorbidity of autism [Bailey et al. 1998], will be reduced. Investigations showed that the GABAergic system was the only one of four investigated neurotransmitter systems (i.e., the GABAergic, serotonergic, cholinergic, and glutamatergic system) found to be reduced in the autistic hippocampus [Blatt et al. 2001]. In addition, Fatemi and colleagues showed a ~50% reduction in protein levels of the enzymes that synthesize GABA in autistic parietal and cerebellar cortices [Fatemi et al. 2002]. Finally, genetic studies implicate GABA receptor involvement in autism [Buxbaum et al. 2002;Jiang et al. 1999;Shao et al. 2003]. Despite the accumulating evidence of the involvement of the GABAergic system in autism, no pharmacological treatment enhancing the availability of the GABAergic system has been developed, (yet).

Cholinergic system: It has been shown that neonatal lesions to basal forebrain cholinergic afferents result in delayed cortical neuronal development and permanently altered cortical cytoarchitecture and cognitive behaviors [Hohmann and Berger-Sweeney 1998], all characteristics of autism [Deuel 2002;Kemper and Bauman 1993;Zilbovicius et al. 1995]. In autism, decreased muscarinic and nicotinic receptor binding was found, indicating abnormal postsynaptic cholinoceptive function [Lee et al. 2002;Perry et al. 2001]. Although the underlying causes of the decrease in nicotinic receptor binding is not understood, it is known that nicotine enhances several cognitive and psychomotor behaviors [Granon et al. 2003], suggesting the potential for intervention through cholinergic receptor modulation. However, no such studies have been performed to date.

Endogeneous opioid system: In 1979, based on the observation that the behavior of animals injected with exogenous opioids was very much similar to autistic behavior (especially socioemotional blindness), Panksepp suggested that symptoms of autism may result from excessive brain opioid activity [Panksepp 1979]. This hypothesis implicated that an opioid antagonist, such as naltrexone, would improve autistic behavior. Although some studies -mostly with small sample sizes- reported positive effects of naltrexone on social behavior or more generally, on the severity of autistic symptoms [Campbell et al. 1989;Kolmen et al. 1995;Leboyer et al. 1992;Lensing et al. 1992;Panksepp and Lensing 1991;Walters et al. 1990], other studies, especially controlled trials in larger

samples of autistic children, failed to show significant improvements in social behavior with naltrexone treatment [Campbell et al. 1993;Willemsen-Swinkels et al. 1995;Willemsen-Swinkels et al. 1996]. However, reductions in self-injurious behavior, hyperactivity, aggressiveness, stereotyped and ritualistic behavior, and attentional dysfunction were found [Barrett et al. 1989;Campbell et al. 1989;Campbell et al. 1993;Chabane et al. 2000;Deutsch 1986;Herman et al. 1987;Kolmen et al. 1995;Kolmen et al. 1997;Sandman 1988;Willemsen-Swinkels et al. 1995;Willemsen-Swinkels et al. 1996](for extensive reviews see [Aman and Langworthy 2000;Riddle et al. 1999]).

Thus, although a lot of effort has been put in the field of neuropharmacology, attempts to develop drugs that specifically improve social and communicative functioning have failed. However, the complicating symptoms of autism such as affective instability, irritability, hyperactivity, inattentiveness, aggression, and self-injury can be successfully treated with medication. The medication-refractory status of social and communicative deficits can be explained on the one hand by the as yet unidentified neurochemical basis of autism, and on the other hand by the obvious lack of involvement of the main neurotransmitter systems in the pathophysiology of social and communicative behavior [Buitelaar 2003].

Overall, converging evidence from several domains of autism research points toward the involvement of widespread abnormalities in brain structures and functions in autism. As autism is such a heterogeneous disorder, searching for the underlying causes is like looking for a needle in a haystack. One of the most important things to do in future research might thus be decreasing sample heterogeneity, which will increase the power to detect brain abnormalities, specific to autism. Defining the underlying brain abnormalities –and its causes- will certainly help in developing better, maybe even curative, (pharmacological) treatments of autism.

In the present thesis we use structural MRI techniques to investigate brain abnormalities in homogeneous groups of high-functioning, medication-naive children, adolescents, and young adults with autism, and their parents. In addition, design-based, observer independent stereology techniques are used to better understand the underlying cellular changes that may cause the brain abnormalities in autism.

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Chapter 2

Review on structural neuroimaging findings in autism

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Abstract

Autism is now widely viewed as a neurodevelopmental disorder, although the underlying biological causes remain to be established. In this review, we examine the literature in magnetic resonance imaging (MRI) as applied to autism, discuss the findings that have emerged, and give directions for potential future research. To date, structural MRI results are inconsistent, partly due to the heterogeneity of the disorder itself, and partly due to the different composition and the varied degree of matching of the studied groups. However, recent studies have begun to elucidate the underlying neuroanatomical abnormalities and brain-behavior relationships in autism, with the most consistent finding being increased brain volume in autism. Future large-scale longitudinal structural imaging studies, starting at very young ages, investigating homogeneous groups of patients and extensively matched control groups, and making use of (combinations of) newer and more sophisticated techniques, hold a great promise to further elucidate the enigma of autism.

Introduction

In 1943, Leo Kanner was the first to introduce the term autism. He posed that ‘‘The outstanding, "pathognomic", fundamental disorder is the children's inability to relate themselves in the ordinary way to people and situations from the beginning of life’’ [Kanner 1943]. The main symptoms of infantile autism, as described by Kanner, can still be found in the diagnostic criteria of the DSM-IV [American Psychiatric Association 1994] and in the Autism Diagnostic Interview [Lord et al. 1994]: extreme autistic aloneness, failure to assume at any time an anticipatory posture, excellent rote memory, delayed echolalia, literalness, repetition of personal pronouns just as heard, all-powerful need for being left undisturbed, monotonously repetitious performances, anxiously obsessive desire for the maintenance of sameness, limitation in the variety of spontaneous activity, and the relation to people is the same as with objects.

As a result of several epidemiological studies, autism is now considered as a predominantly genetically determined neurodevelopmental disorder. First, the concordance rate for monozygotic twins is rated between 60% [Bailey et al. 1995] and >90% [Ritvo et al. 1985;Steffenburg et al. 1989], whereas the concordance rate in dizygotic twins is only between 0% and 24%. Second, the recurrence risk for siblings is approximately 3-5%, which corresponds to an incidence 100 times greater than in the normal population [Skuse 2000]. Third, 70% of all autistic children have mental retardation and about 60% has epilepsy [Bailey et al. 1996;Yeargin-Allsopp et al. 2003]. In addition, in 6% of cases, neurological diseases such as tuberous sclerosis, neurofibromatosis, fragile X syndrome, and phenylketonuria may be associated with autistic features [Fombonne 1999]. Presently, autism is considered to be the most strongly genetically influenced childhood psychiatric disorder [Andres 2002;Bailey et al. 1995;Bolton et al. 1994;Lauritsen and Ewald 2001;Liu et al. 2001;Skuse 2000;Trottier et al. 1999;Turner et al. 2000], with an estimated heritability of more than 90% [Bailey et al. 1996] defined by the presence of marked social deficits, specific language abnormalities, and stereotyped, repetitive behaviors [American Psychiatric Association 1994]. Behavioral abnormalities are typically observed before the age of 30 months is reached and persist throughout life, although symptoms generally become less severe with age [Korkmaz 2000;Rumsey et al. 1985]. In a review of 23 epidemiological surveys of autism, published between 1966 and 1998, the median prevalence estimate of autism was 5.2/10000, whereas the minimum estimate for all forms of pervasive developmental disorders was 18.7/10000 [Fombonne 1999], with boys approximately four times more affected than girls [Ciaranello and Ciaranello 1995;Lord et al. 1994;McLennan et al. 1993;Szatmari et al. 2000;Yeargin-Allsopp et al. 2003]. Although no evidence for a secular increase in the incidence can be found, the prevalence for both autism and related pervasive developmental disorders

appear to increase with time [Bertrand et al. 2001;Chakrabarti and Fombonne 2001;Charman 2002;Fombonne 2003;Kadesjo et al. 1999;Yeargin-Allsopp et al. 2003], probably due to changes in case definition and improved recognition.
From the early 1970s on, researchers started to investigate the underlying substrates and mechanisms, causing autism. To date, an accumulating body of evidence suggests that head and brain size in autism is, on average, increased. Head circumference studies showed macrocephaly, defined as head circumference above the 97th percentile, in approximately 20% of subjects with autism [Aylward et al. 2002;Bailey et al. 1993;Davidovitch et al. 1996;Fidler et al. 2000;Fombonne 2000;Lainhart et al. 1997;Miles et al. 2000;Naqvi et al. 2000;Stevenson et al. 1997;van Karnebeek et al. 2002;Woodhouse et al. 1996], a finding that had already been noticed by Kanner in 1943 [Kanner 1943]. This macrocephaly is not present until the first few years of life [Courchesne et al. 2001;Lainhart et al. 1997;Stevenson et al. 1997], suggesting an underlying mechanism that involves a decrease in elimination processes -such as apoptosis and pruning- during early development.
Consistent with these clinical findings, neuropathological studies have reported increased brain weight in autistic individuals [Bailey et al. 1998;Casanova et al. 2002;Courchesne et al. 1999;Kemper and Bauman 1998].
With the invention of neuroimaging techniques, especially Magnetic Resonance Imaging (MRI), in vivo examination of brain structures and functions was made possible. Important findings in the MRI literature are now beginning to emerge, which will help in elucidating the underlying neurodevelopmental mechanisms and brain-behavior relationships in autism. Characterization of the anatomical and functional brain phenotypes may, in turn, contribute to clarification of the genetic basis by providing insight into the brain phenotypes. The present review will be discussing structural abnormalities in autism detected with MRI.

Neuroimaging:

Imaging the anatomical substrate of autism

Computerized Tomography (CT): The first in vivo studies of brain anatomy in autism used Computerized Tomography (CT), which has a poor spatial resolution compared to Magnetic Resonance Imaging (MRI). As a consequence, qualitative ratings were often used as measurements [Campbell et al. 1982;Caparulo et al. 1981;Creasey et al. 1986;Damasio et al. 1980;Gillberg and Svendsen 1983;Hier et al. 1979;Prior et al. 1984;Tsai et al. 1982], although a few quantitative CT studies have been performed [Jacobson et al. 1988;Rosenbloom et al.

1984]. Moreover, the use of ionizing radiation raised the ethical problem of repeated scanning and inclusion of healthy controls. Findings from these CT studies are not as informative as the later results from studies using MRI.
Magnetic Resonance Imaging (MRI): MRI of brain structures is uniquely suited to map the neuroanatomical abnormalities that characterize autism, without the use of ionizing radiation. It therefore permits (repeatedly) scanning of (young) children.

Structual imaging studies

Early structural studies of autism were hampered by a number of methodological shortcomings, making it hard to interpret the reported findings: small sample sizes; the use of patients with associated medical and neurological conditions; the use of "medical" controls; no matching on confounding factors, such as age, sex, IQ, and medication status; low power of the magnetic field strength (0.5 Tesla) and thick slices (>1.5 mm). Fortunately, in the last decade, most studies investigated more homogeneous groups of patients and more extensively matched control groups and used more sophisticated MRI techniques. Quantitative MRI studies on autism are summarized in table 1 and will be discussed below.

Total brain volume

In 1992, the first quantitative MRI study investigated 9 children with high-functioning autism (HFA), 13 children with low-functioning autism (LFA), 15 children with developmental language disorder (DLD), 10 children with mental retardation (MR), and 24 control children [Filipek et al. 1992]. All children were between 6 and 10 years of age. A hierarchy was found for almost all global measurements (except the hippocampus and the ventricles), with HFA > LFA > DLD > C > MR. Likewise, Piven et al. reported increased brain volume and increased lateral ventricular volume in 22 adult males with autism, compared to 20 adult male controls [Piven et al. 1995]. This brain enlargement remained significant after correction for height and performance IQ. A year later, Piven et al. replicated their findings in a larger sample of 35 adults with autism (26 males) and 36 adult controls (20 males), including the original sample of their previous study [Piven et al. 1996]. In addition, they reported that the increased brain volume in autistic individuals was only present in males, not in females, and was confined to the parietal, temporal, and occipital, but not the frontal, lobe. Independently, Hardan et al. reported increased brain and third ventricular volume after adjustment for intracranial volume in a sample of 16 male adults with HFA, compared to 19 male controls [Hardan et al. 2001a]. Likewise, Sparks et al. found increased total brain volume in 45 subjects with autism spectrum disorder (ASD) compared to 26 controls, although the subjects' age range (3-4 years of age) differed significantly from earlier studies [Sparks et al.

2002]. Recently, Herbert et al. reported an overall increase in brain volume in 15 boys with HFA compared to 17 control boys [Herbert et al. 2003]. More detailed analysis revealed three groupings of brain structures. One grouping, including thalamus, cerebellum, globus pallidus-putamen, and brainstem, showed enlargement, proportional to the increase in brain volume. The second grouping, including only cerebral white matter, was significant larger, both before and after correction for brain volume. The third grouping, including cerebral cortex and the amygdala-hippocampal complex showed comparable volumes between autistic boys and control boys before correction for brain volume, but were significantly smaller after correction for brain volume. However, it should be mentioned, that only age range (7-11 years) and minimum IQ (80) was given, without any information on matching. Furthermore, in contrast to the histogram based gray-white separation [Schnack et al. 2001], intensity contour algorithms were used for separation of gray and white matter, which can be quite difficult in low contrast transitional areas. In contrast to the studies by Piven et al. and Hardan et al., three studies (two of them by the same group) claimed that increased brain volume was only present in (very) young children [Aylward et al. 2002;Carper et al. 2002;Courchesne et al. 2001]. Aylward et al. investigated 67 subjects with HFA between 8 and 46 years of age and 83 thoroughly matched controls and reported increased head circumference in patients among all age groups [Aylward et al. 2002]. In contrast, increased brain volume was only present in autistic children 12 years of age and younger. The study by Courchesne et al. (60 autistic and 52 control children) reported increased brain volume, due to both increased cerebral gray and white matter volume, only in 2-3 year old autistic children, not in 4-16 year olds [Courchesne et al. 2001]. In addition, Carper et al., investigating a subset of the Courchesne sample (38 autistic and 39 control children), specified the earlier reported increased cerebral gray and white matter volume and found an increase in frontal and temporal gray matter and in frontal, parietal and temporal white matter volume, again only in the 2-3 year old autistic children [Carper et al. 2002]. However, it should be noted that in these last two studies the patient and the control group were not matched on IQ. Moreover, in the patient group, but not in the control group, subjects with mental retardation were included. Likewise, two other studies, failing to find brain enlargement in autism [Rojas et al. 2002;Townsend et al. 2001], did not control for the significantly lower IQ in the autism groups. Townsend et al. examined 9 male adults with autism and 14 age-matched male controls and found no difference in brain volume, gray matter or white matter volume between the two groups [Townsend et al. 2001]. Rojas et al. studied 15 adults with autism (13 males) and 15 adult controls (13 males) and did not find any difference in hemisphere volumes [Rojas et al. 2002]. Finally, two studies have been performed, including only patients with Asperger's syndrome (AS)[McAlonan et al. 2002;Murphy et al.

2002]. Neither of these two studies found any difference in total brain volume between subjects with AS and controls. McAlonan et al. included 21 adults with AS (19 males) and 24 adult controls (22 males) of comparable IQ and age [McAlonan et al. 2002]. No significant difference in total brain, lobar, caudate nucleus, putamen, cerebellum or ventricular volume was found between the two groups. However, using voxel-based morphometry, local gray matter deficits were found in the fronto-striatal and cerebellar regions, whereas white matter deficits were found in the left hemisphere, pons and cerebellum. White matter excesses, on the other hand, were found around the basal ganglia. Furthermore, it was reported that, with increasing age, brain volumes became smaller in controls, but no such relationship was found in the AS sample. The other Asperger's syndrome study, by Murphy et al., included 14 adult males with AS and 18 adult male controls [Murphy et al. 2002]. As this study was primarily performed to measure brain metabolites, only two voxels of interest were measured –one of 8.5 mL in the right medial prefrontal lobe and one of 8.5 mL in the right medial parietal lobe. No volumetric differences between the two groups were found in these two regions.

In summary, the results of studies on brain size in autistic subjects are rather inconclusive. It is known that sex [Caviness et al. 1996;Giedd et al. 1996a], age [Caviness et al. 1996;Giedd et al. 1996a], IQ [Andreasen et al. 1993;Posthuma et al. 2002;Thompson et al. 2001], handedness [Witelson and Goldsmith 1991], length [Piven et al. 1996], weight, socioeconomic status [Andreasen et al. 1990] and the use of (neuroleptic) medication [Chakos et al. 1994;Chakos et al. 1998;Scheepers et al. 2001] are correlated to the size of several brain structures. Thus, differences in composition of the patient groups or non-matching between patients and controls on any of these variables could have confounded the results. Furthermore, relatively small sample sizes may have caused type II error, due to lack of statistical power. However, taking into account those studies, controlling for confounding variables, convergent evidence of brain enlargement in autism was found. However, the issue seems to be as to what age this enlargement is present. Some studies suggest that brain enlargement in autism is restricted to early childhood [Aylward et al. 2002;Courchesne et al. 2001], whereas other studies report brain enlargement still present in adulthood [Hardan et al. 2001a;Piven et al. 1995;Piven et al. 1996]. Of interest is the fact that studies reporting increased brain volume in adulthood included only high-functioning patients, contrary to those studies who did not, but see [Aylward et al. 2002]. Longitudinal studies –both studies with low-functioning and studies with high-functioning subjects- will be necessary to clarify the timing and duration of the brain enlargement in autism and may reveal different patterns of brain growth in HFA and in LFA.

Cerebellum

The fundamental function of the cerebellum is to learn predictive relationships among sequences of events so that whenever an analogous sequence begins to unfold in real time, the cerebellum can generate predictions about upcoming events and prepare whichever neural systems are expected to be needed to respond appropriately to such information [Allen and Courchesne 2003].

The first cerebellum studies measured midsagittal areas and reported smaller cerebellar hemispheric areas [Gaffney et al. 1987a;Murakami et al. 1989]. Looking at the cerebellar vermis, contradictory findings on area measurements of the cerebellar vermis, particularly lobules VI-VII were reported. Few studies reported hypoplasia of lobules VI-VII [Ciesielski et al. 1997;Courchesne et al. 1988], or entire cerebellar vermis [Hashimoto et al. 1995]. Note that these studies did not account for differences in IQ, a factor known to be of influence on brain structure. Later on, Courchesne et al. specified their findings, reporting two subgroups in autism, one with hypoplasia and one with hyperplasia of vermal lobules VI-VII [Courchesne et al. 1994c;Courchesne et al. 1994a; Courchesne et al. 1994b], but one has to bear in mind that groups were not matched on IQ. On the other hand, numerous studies failed to find any differences in vermis lobules VI-VII [Filipek et al. 1992;Garber and Ritvo 1992;Hashimoto et al. 1992;Hashimoto et al. 1993a;Hashimoto et al. 1993b; Holttum et al. 1992;Kleinmand et al. 1992;Nowell et al. 1990;Piven et al. 1992;Piven et al. 1997a;Ritvo and Garber 1988]. It was suggested [Piven et al. 1992] that methodological issues, especially not controlling for the confounding variable IQ, was a major potential source of error in some studies [Courchesne et al. 1988;Courchesne et al. 1994c;Courchesne et al. 1994b;Courchesne et al. 1994a].

Moreover, while hypoplasia of lobules VI-VII was not replicated, studies measuring cerebellar volume, reported increased volume, both in autistic children [Herbert et al. 2003;Sparks et al. 2002], and in adults with autism [Hardan et al. 2001b;Piven et al. 1997a]. Both Herbert et al. and Sparks et al. reported increased cerebellar volume, proportional to the increase in brain volume, respectively in 15 boys with HFA and 17 control boys between 7 and 11 years of age [Herbert et al. 2003] and in 45 children with ASD between 3 and 4 years of age, compared to 26 age-matched control children [Sparks et al. 2002]. Likewise, Piven et al. found increased cerebellar volume, in a group of 35 adults with autism (26 males), when compared to a group of 36 adult controls (20 males) [Piven et al. 1997a]. The significant difference in cerebellar volume disappeared after correction for brain volume, implying that the increase in cerebellar volume was proportional to the increase in brain volume. Hardan et al. reported increased cerebellar volume in 16 adult males with autism compared to 19 age- and IQ-matched male controls [Hardan et al. 2001b]. The cerebellar enlargement remained significant after correction for brain volume.

No difference in vermal lobules VI-VII was found. Two other studies, both by the Courchesne group, did not find cerebellar enlargement in older autistic children and adults [Allen and Courchesne 2003;Courchesne et al. 2001]. Courchesne et al. did report enlargement in cerebellar volume, due to increased white matter volume in 2-3 year old autistic children, but not in 4-16 year olds [Courchesne et al. 2001]. Allen et al. studied 8 adult subjects with autism (7 males) and compared them to 8 age-, sex-, and handedness-matched adult controls (7 males). The cerebellum was found to be smaller in the autistic group, although this difference did not reach significance [Allen and Courchesne 2003]. However, it should be noted that in both studies, the patient and the control group were not matched on IQ.
In conclusion, it can be assumed that, when controlling for confounding variables, especially IQ, autism is associated with increased cerebellar volume.

Basal ganglia

The basal ganglia, consisting of the caudate nucleus, putamen, and globus pallidus, are believed to be involved in stereotyped en repetitive behavior, as seen in autism. Indeed, Sears et al. reported increased caudate nucleus volume, proportional to the increase in brain volume, in 35 adults with HFA (26 males) compared to 36 adult controls (20 males) of comparable age, sex, and IQ [Sears et al. 1999]. The increase in caudate nucleus volume was positively correlated with the ADI subitem "complex repetitive motor behaviors" and negatively with the ADI subitems "compulsions and rituals" and "difficulties with minor change in environment and routine" in autism. In the same article, the authors replicated their own findings in an independent sample of 13 autistic subjects and 25 controls, comparable for age and performance IQ. In this second study, three contiguous sagittal slices containing the caudate nucleus, were traced. Again, caudate nucleus was significantly larger in the patient group, compared to the control group. Finding such brain-behavior relationships will be useful in dissecting the heterogeneity of autism and may be helpful in finding more homogeneous subgroups. However, it speaks for itself that these findings need replication. More recently, Herbert et al. studied the volumes of the basal ganglia in 15 boys with HFA and 17 control boys [Herbert et al. 2003]. Results were in contrast to the Sears et al. study, as Herbert et al. found an increase in globus pallidus-putamen, proportional to the increase in brain volume, but no differences in the caudate nucleus.
As only two studies, with conflicting results, have investigated the basal ganglia in autism, more research is definitely needed before statements can be made about volumetric abnormalities in the basal ganglia.

Thalamus

The thalamus is a central structure in almost all neural systems, with reciprocal connections to virtually every major region of the brain [Katz and Shatz 1996]. Thus, the thalamus is believed to be implicated in attention, memory, language, and emotional processing, all areas known to be deficient in autism. Recently, increasing interest in the cerebello-thalamo-cortical pathway has been shown in autism [Carper and Courchesne 2000;Chugani et al. 1997;Hardan et al. 2001b]. However, to date, only two studies investigated the thalamus with sMRI [Herbert et al. 2003;Tsatsanis et al. 2003]. Tsatsanis et al. compared 12 adult males with HFA with 12 adult male controls, matched on age and IQ. No significant difference in unadjusted thalamic volumes was found. However, a significant difference in correlation between thalamic volume and brain volume was reported. A positive correlation between thalamic and brain volume was found in controls, whereas no such correlation was found in the autism group. When dividing both groups in a "larger brain" and a "smaller brain" group, significantly smaller thalamic volumes were found in the autism "larger brain" group compared to the control "larger brain" group. It was suggested that smaller thalamic volumes might result in fewer axons. As the thalamus affects the functional differentiation of the cortex very early in development, decreased, or even absent, thalamic input might result in disruption of cortical development and underdeveloped connections between cortical and subcortical regions. On the other hand, Herbert et al. reported increased thalamic volume before, but not after correction for brain volume in 15 boys with HFA, when compared to 17 control boys [Herbert et al. 2003]. Thus, future studies with larger sample sizes are needed in order to determine the possible volumetric abnormalities of the thalamus in autism.

Corpus Callosum (CC)

The CC is the main transverse fiber tract that connects most, but not all areas of the two cerebral hemispheres. It is involved in interhemispheric transfer of information and therefore has become important in studying cortical connectivity in the brain. Based on both functional [Zilbovicius et al. 1995] and neuropsychological [Minshew et al. 1997] studies, cortical connectivity of the brain is suggested to be abnormal in autism, implying that the CC might be abnormal as well. In 1987, the first study investigating the midsagittal CC reported a smaller CC in 13 children with HFA (10 males) in comparison to 35 medical control children (21 males), although this difference did not reach significance [Gaffney et al. 1987b]. However, it should be noted that several factors could have confounded the results. The study was performed on a 0.5 Tesla scanner, providing less clear images than the more modern 1,5 Tesla scanners. Furthermore, medical controls, with unknown IQ, were used as comparison group. Following this initial study of the CC, all other studies examining

the CC reported smaller midsagittal CC, [Egaas et al. 1995;Hardan et al. 2000;Manes et al. 1999;Piven et al. 1997b;Saitoh et al. 1995], but see [Elia et al. 2000]. Saitoh et al. reported a smaller CC in 33 autistic individuals (30 males; 12 subjects with mental retardation) compared to 23 controls (19 males) [Saitoh et al. 1995]. The CC was divided into 5 regions, and the reduction in CC was found in the posterior regions, where the parietal fibers are concentrated. Likewise, Egaas et al. found reduction of the CC in 51 autistic subjects (45 males; 16 subjects with mental retardation) compared to 51 age-, sex, and handedness-matched controls (45 males) [Egaas et al. 1995]. Again, the CC was divided into five regions and the most significant reduction was found in the posterior CC. Two years later, Piven et al. divided the CC in three regions (anterior, body, and posterior) and reported smaller CC in 35 subjects with autism (26 males) compared to 36 controls (20 males) [Piven et al. 1997b]. After adjustment for sex, performance IQ, and total brain volume, both the body and the posterior part of the CC were significantly smaller in the autism group. The authors posed two possible explanations for the decrease in posterior CC. First, as they had previously reported increased parietal lobe volume [Piven et al. 1996], they suggest that local, ipsilateral cortical connections outcompete more distant, contralateral connections. Alternatively, the relatively smaller CC could be due to a greater volume of nonneuronal cortical tissue or cortical neurons that do not project axons to the CC. In 1999, Manes et al. investigated the CC in a group of mentally retarded autistic individuals [Manes et al. 1999]. The CC was divided in 7 subregions and comparison was made between 27 mentally retarded autistic subjects (22 males) and 17 controls (11 males), of comparable age, performance IQ, weight, and height. The CC was reported to be significantly smaller in the autism group. Dividing the CC in its subregions, all regions, except the most anterior (rostrum) and most posterior (splenium) regions were found to be smaller. The authors raise the issue of the underlying mechanism of the smaller CC in autism. Either hypoplasia or atrophy is suggested as causal mechanism. However, to date, no definitive answer is possible, as no longitudinal MRI studies of the CC have been performed. The most recent study, reporting decreased CC in autism, investigated 22 adult males with HFA and 22 individually age-, sex-, and IQ-matched controls [Hardan et al. 2000]. The CC was divided in 7 subregions and a significant reduction in anterior CC was found, remaining significant after correction for intracranial volume, total brain volume, and white matter volume. In addition, an overall reduction in CC was found, but this reduction did not reach significance ($p = 0.08$). Although contrary to earlier studies -reporting smaller *posterior CC*- the finding of a smaller anterior CC was not unexpected, as the prefrontal cortex –known to be delayed in development [Zilbovicius et al. 1995]- projects to the *anterior CC*. Fewer projections from the prefrontal cortex may result in a smaller anterior CC. Finally, one recent study failed to find CC differ-

ences between 22 male subjects with LFA and 22 age- and sex-matched controls [Elia et al. 2000]. However, it should be noted that this study was performed on a 0.5 Tesla scanner and no IQ measures were reported.
In conclusion, rather consistent results of decreased CC volume, especially the posterior CC, have been reported. The discrepant findings of increased brain volume and decreased CC volume may provide clues as to which cortical layers may be implicated, i.e. only cortical layer III contributes axons that cross the CC.

Medial temporal lobe structures

Amygdala: The amygdala is implicated in social behavior and cognition [Adolphs 2001;Adolphs et al. 2002;Adolphs 2002]. As one of the core dysfunctions of autism is social impairment, the amygdala was suggested to be abnormal, either in structure and/or in function in autism. To date, several studies have investigated amygdala volumes in autism, with contradictory findings. Increased [Abell et al. 1999;Howard et al. 2000;Sparks et al. 2002], decreased [Aylward et al. 1999;Herbert et al. 2003;Pierce et al. 2001] and "normal" amygdala volumes [Haznedar et al. 2000] have been reported. Using voxel-based morphometry, Abell et al. investigated 15 adults with HFA (12 males) and 15 age- and IQ-matched controls (12 males) [Abell et al. 1999]. Increased gray matter was found in amygdala and peri-amygdaloid cortex. Howard et al. reported bilateral increased amygdala volume [Howard et al. 2000]. In their study, amygdala volume and social perception were examined in 10 male adults with HFA and 10 age-, sex-, and verbal IQ-matched controls. Although no direct correlations between amygdala volumes and performance on social perception were calculated, subjects with HFA (1) made more errors in recognition of fear, but not in recognition of other basic emotions (happiness, surprise, sadness, disgust, and anger), (2) were impaired in perception of eye-gaze direction, and (3) scored lower in a facial recognition memory task. It was suggested that increased amygdala volume might indicate sub-optimal operation of this structure, causing impairments in social perception. Furthermore, it was hypothesized that incomplete neuronal pruning might have caused the increased amygdala volume. The third study reporting bilateral increased amygdala volume studied 45 subjects with ASD between 3 and 4 years (38 males) of age and 26 age-matched controls (18 males) [Sparks et al. 2002]. The increase in amygdala volume was found to be proportional to the increase in brain volume. However, in the group with the most severely affected children (the core autism group), amygdala enlargement was disproportional to the increased brain volume, implying a positive correlation between amygdala volume and severity of symptoms. Contrary to these reports of increased amygdala volume, three studies [Aylward et al. 1999;Herbert et al. 2003;Pierce et al. 2001] reported decreased amygdala volume in autism. Aylward et al. studied 14

male adolescents and adults with HFA and 14 controls, matched on age, sex, IQ, height, weight, and socioeconomic status [Aylward et al. 1999]. Both with and without correction for total brain volume, amygdala volumes were significantly smaller in the autism group. It was suggested that these results are consistent with post-mortem findings of increased cell packing density of small, immature-appearing neurons with truncated dendritic development [Kemper and Bauman 1998]. Likewise, Pierce et al. reported bilateral decrease in amygdala volume in 7 male adults with HFA compared to 8 male adults controls, individually matched on age, sex, and handedness (no IQ was mentioned for the control subjects) [Pierce et al. 2001]. In addition, a trend was found for correlation between smaller left amygdala volume and less activation in the amygdala during face processing. However, it should be noted that, besides the small sample size, the amygdala volumes reported for control subjects were much smaller than normally reported with the anatomical criteria used (Watson's criteria) [Brierley et al. 2002], which might indicate that not the whole amygdala was traced. Herbert et al. reported decreased amygdala volume in 15 boys with HFA compared to 17 control boys, only after correction for brain volume [Herbert et al. 2003]. However, Herbert et al. combined the amygdala and the hippocampus and no separate volumes of the amygdala were measured. Finally, one study reported no difference in amygdala volume between 17 adults with ASD (10 autism and 7 AS) (15 males) and 17 adult, age- and sex-matched controls (15 males) [Haznedar et al. 2000]. It should be noted that the patient group included subjects with mental retardation (IQ range 55-125), which was not the case in the control group (IQ range 88-136). Although no volumetric differences were found, the patient –but not the control- group showed a positive correlation between amygdala volume and semantic categorization scores, performed during PET scanning. Interestingly, when dividing the patient group in autism and Asperger syndrome, the subjects with Asperger's syndrome –but not patients with autism- appeared to have significantly larger left amygdala volumes compared to controls. This enlargement of the left amygdala was negatively correlated with scores on the ADI nonverbal communication subscale.

In summary, although results are equivocal, the majority of studies report amygdala volume increases. Unfortunately, to date, very little attention was paid to the possible correlations between amygdala volume and social cognition, which is certainly warranted in future studies.

Hippocampus: The hippocampus is implicated in associative memory and plays a critical role in binding together multiple inputs to permit representations of the relations among the constituent elements of scenes or events [Cohen et al. 1999]. Like in amygdala studies, no consistent abnormalities have been reported. Increased [Sparks et al. 2002], decreased [Aylward et al. 1999;Herbert et al. 2003;Saitoh et al. 2001], or "normal" hippocampal [Haznedar et al. 2000;Howard et al. 2000;Piven et al. 1998;Saitoh et al. 1995] volumes have

been reported in autism. The first study investigating the hippocampus in autism measured the posterior hippocampal formation (CA1 – CA4, dentate gyrus, subiculum) in one single oblique coronal slice [Saitoh et al. 1995]. No differences in hippocampal area were found between 33 autistic children (12 with mental retardation) (30 males) and 23 control children (19 males), regardless of whether the autistic subjects were retarded or not or whether they had a history of seizures or not. Two years later, Piven et al. replicated this finding, reporting no volumetric differences in hippocampus in 35 autistic subjects (26 males) compared to 36 controls (20 males), neither before, nor after adjustment for sex, performance IQ and total brain volume [Piven et al. 1998]. Two other studies failed to find differences in hippocampal volume, investigating 17 adults with ASD (10 autism and 7 AS) (15 males) and 17 adult, age- and sex-matched controls (15 males) [Haznedar et al. 2000] and 10 male adults with HFA and 10 age-, sex-, and verbal IQ-matched controls [Howard et al. 2000], respectively. However, in the last study, a trend towards smaller hippocampus was found in the autism group ($p=0.068$). Contrary to the "negative" studies, a significant decrease in hippocampal volume in autism was reported three times [Aylward et al. 1999;Herbert et al. 2003;Saitoh et al. 2001]. In 1999, Aylward et al. studied hippocampal volume in 14 male adolescents and adults with HFA and 14 male controls, matched on age, IQ, length, weight, and socioeconomic status [Aylward et al. 1999]. Hippocampal volume appeared to be smaller in autistic subjects, but only after correction for total brain volume, which was slightly larger in the autism group. Similar to the smaller amygdala reported in this study (see above), these findings were suggested to be in accordance with post-mortem reports of increased cell packing density of small, immature-appearing neurons [Kemper and Bauman 1998]. The hippocampal volume reduction was less pronounced than the reduction in amygdala volume, implying that the amygdala has a more essential role than the hippocampus in the pathophysiology of the behavioral features of autism. Likewise, Herbert et al. reported smaller hippocampal volume after correction for brain volume in 15 boys with HFA compared to 17 control boys [Herbert et al. 2003]. However, as was mentioned before, combined volumes of amygdala and hippocampus were measured, no separate volumes of the hippocampus. Saitoh et al. reported smaller area dentata (dentate gyrus and CA4), both before and after correction for total brain volume in 59 autistic children (52 males) compared to 51 controls (40 males) [Saitoh et al. 2001]. No differences were found in CAS (subiculum and CA1-CA3). However, it should be noted that cross-sectional areas were measured, no volumes. The authors state that these findings are compatible with post-mortem findings, reporting smaller pyramidal neurons in CA4, but not in CA1, while dendritic branching was decreased in both regions [Raymond et al. 1996]. To date, only one study reported increased hippocampal volume in 45 children with ASD between 3 and 4 years of age (38 males) compared to 26 age-matched control children (18 males) [Sparks et al. 2002]. This increase was

proportional to the increase in total brain volume. It was suggested that individuals with autism might exhibit arrested development, or increased apoptosis over time, explaining the increased hippocampal volume at 3-4 years of age, disappearing at older ages.
In conclusion, regarding the available data, it is not possible to draw firm conclusions regarding hippocampal volume in autism, partly due to differences in composition of the studied groups.
Regarding the amygdala and the hippocampus, one should pay attention to the papers by Casey et al. [Casey et al. 2000] and Giedd et al. [Giedd et al. 1996b]. The amygdala has a preponderance of androgen receptors, the hippocampus predominantly estrogen receptors. Thus, in the light of the extreme male brain theory, posed by Baron-Cohen [Baron-Cohen 2002], abnormalities would be expected in the amygdala, rather than in the hippocampus.

Cingulate gyrus

The anterior cingulate gyrus is thought to be involved in information processing and response to emotional cues, and is therefore a region of interest in autism. To date, only one study investigated volumes of the anterior cingulate in autism [Haznedar et al. 1997]. Seven adults with HFA (5 males) and 7 age-matched controls (5 males) were studied. The anterior cingulate was divided into three dorsoventral regions (Brodmann's area 25, 24, and 24'). The right area 24' was found to be significantly smaller in the autism group after correction for total brain volume. In addition, this region was less active during a verbal learning test, although these two findings did not show a significant correlation. The authors suggested that, although post-mortem research reported increased cell packing density in this region, rather than cell loss [Bauman and Kemper 1994], reduced dendritic fields could be associated with cells packed more closely together, and hence resulting in smaller total structure. However, future studies investigating the cingulate gyrus are definitely needed.

Planum temporale

The structure of the planum temporale (PT) (roughly consistent with Wernicke's area) is often abnormal in disorders with associated communication problems. Hence, the PT is a structure of interest in autism. Heschl's gyrus (HG) is another structure, worth studying, as it has a role in auditory processing. To date, one study investigated the PT and the Heschl's gyrus (HG) in autism [Rojas et al. 2002]. Fifteen adults with autism (13 males) were compared to 15 controls (13 males), matched on age and handedness, although the autism group had significantly lower IQ's than the control group. No differences in HG were found, but a significantly smaller left PT was reported in the autism group. This volume reduction in left PT may suggest an early neurodevelopmental disturbance in autism that impacts language development. However, no definite conclusion can be drawn from this one single study.

Table 1: Articles of structural MRI in autism

Region	Author	Journal	Published	Design Technique N; gender; mean age (or range) (measures of interest)	Results
TBV	Herbert et al.	Brain	2003;126:1182-1192	SMRI 15 A;17m;7-11y 17 C; 17m;7-11y TBV, GM, WM, CB, basal ganglia, thalamus, AMY, HIP	Trend towards ↑ TBV; ↑WM before and after correction TBV; ↓ GM after correction TBV
	Aylward et al.	Neurology	2002;59:175-183	SMRI 67 A;58m;18.8y 83 C;76m18.9y HC and TBV	↑ HC in all ages (8-46y); ↑TBV only in ≤ 12y
	Carper et al.	Neuroimage	2002;16:1038-1051	SMRI 38 A;38m;5.7y 39 C;39m;6.5y TBV, GM/WM; cortical lobes	↑ FR, PA and TE WM and FR and TE GM only in 2-3y
	McAlonan et al.	Brain	2002;125:1594-1606	SMRI 21 AS;19m;32y 24 C;22m;33y TBV; CB; vent; cortical lobes; caudate; putamen	No differences in brain volumes; ↓ in brain volumes with age in C, but not in AS; locally ↓ GM fronto-striatal and CB, ↓ WM left hemisphere, ↑ WM around basal ganglia
	Murphy et al.	Arch. Gen. Psychiatry	2002;59:885-891	SMRI 14 AS;14m;30y 18 C;18m;32y FR and PA GM/WM; CSF	No differences in brain volumes
	Rojas et al.	Neurosci. Lett.	2002;328:237-240	SMRI 15 A;13m;29.9y 15 C;13m;30.4y left and right hemisphere	No difference in volumes of hemispheres
	Sparks et al.	Neurology	2002;59:184-192	SMRI 45 ASD;38m;4y 14 DD;6m;4y 26 C;18m;4y TBV, CB, AMY, HIP	↑ TBV
	Courchesne et al.	Neurology	2001;57:245-254	SMRI 60 A;60m;6.2y 52 C; 52m;2-16y TBV, GM/WM, CB	↑ cortical GM and WM only in 2-3 y olds.

	Hardan et al.	J. Child Neurol.	2001;16:421-424	SMRI 16 A;16m;22.2y 19 C;19m;22.2y IC, TBV, vent	↑ TBV and 3rd vent after correction for IC
	Townsend et al.	Brain Res. Cogn. Brain Res.	2001;11:127-145	SMRI 9 A;9m;28.3y 14 C;14m;26.8y IC, TBV, CSF GM/WM, CB	↑ CSF 7x hypo- and 2x hyperplasia CB lobules VI-VII (4% IC, but NS)
	Piven et al.	JAACAP	1996;35:530-536	SMRI 35 A;26m;18y 36 C;20m;20.2y IC, TBV, CSF, cortical lobes	↑ IC in males; ↑ PA, TE and OC lobe both in males and females
	Piven et al.	Am. J. Psychiatry	1995;152:1145-1149	SMRI 22 A;22m;18.4y 20 C;20m;21.6y IC, TBV, vent	↑ IC, TBV, vent
	Filipek et al.	Ann. Neurology	1992;32:475	SMRI 22 A;?;6-10y 15 DLD;?;6-10y 10 MR;?;6-10y 24 C;?;9.2y TBV, GM/WM, CB caudate, AMY, putamen, HIP, vent, brainstem	A > DLD > C > MR, except for HIP and vent. High IQ A larger volumes than low IQ A
Cerebellum	Herbert et al.	Brain	2003;126:1182-1192	SMRI 15 A157m;7-11y 17 C; 17m;7-11y TBV, GM, WM, CB, basal ganglia, thalamus, AMY, HIP	↑ CB before correction TBV
	Allen et al.	Am. J. Psychiatry	2003;160:262-273	SMRI 8 A;7m;26.9y 8 C; 7m;26.8y CB, GM	NS smaller CB
	Sparks et al.	Neurology	2002;59:184-192	SMRI 45 ASD;38m;4y 14 DD;6m;4y 26 C;18m;4y TBV, CB, AMY, HIP	↑ CB, proportional to ↑ TBV

	Courchesne et al.	Neurology	2001;57:245-254	SMRI 60 A;60m;6.2y 52 C; 52m;2-16y TBV, GM/WM, CB	↑ CB WM only in 2-3 y olds.
	Hardan et al.	JAACAP	2001;40:666-672	SMRI 16 A;16m;22.4y 19 C;19m;22.4y CB	↑ CB
	Piven et al.	Neurology	1997;49:546-551	SMRI 35 A;26m;18y 36 C;20m;20.2y CB	↑ CB
Basal ganglia	Herbert et al.	Brain	2003;126:1182-1192	SMRI 15 A157m;7-11y 17 C; 17m;7-11y TBV, GM, WM, CB, basal ganglia, thalamus, AMY, HIP	↑ globus pallidus-putamen before correction TBV, but not caudate nucleus
	Sears et al.	Prog. Neuropsycho-pharmacol. Biol. Psychiatry	1999;23:613-624	SMRI 35 A;26m;18y 36 C;20m;20.2y caudate, putamen, globus pallidus	↑ caudate associated with compulsions, rituals, difficulties with minor changes, and complex motor mannerisms
Thalamus	Herbert et al.	Brain	2003;126:1182-1192	SMRI 15 A157m;7-11y 17 C; 17m;7-11y TBV, GM, WM, CB, basal ganglia, thalamus, AMY, HIP	↑ diencephalon (= thalamus + ventral diencephalon)
	Tsatsanis et al.	Biol. Psychiatry	2003;53:121-129	SMRI 12 A;12m;21y 12 C;12m;18.1y thalamus	↓ thalamus in those with large TBV
CC*	Elia et al.	J. Child Neurol.	2000;15:504-508	SMRI 22 A;22m;10.9y 11 C;11m;10.9y CC	↓ No difference, but on 0.5 T, with 5 mm slide thickness
	Hardan et al.	Neurology	2000;55:1033-1036	SMRI 22 A;22m;22.4y 22 C;22m;22.4y CC (7 subregions)	↓ anterior CC (rostrum + genu); trend towards overall ↓ in CC
	Manes et al.	J. Neuro-psychiatry Clin. Neurosci.	1999;11:47-477	SMRI 27 A;22m;4.6y^ 17 C;11m;4.5y^ CC (7 subregions)	↓ CC, most marked in the body of the CC

	Piven et al.	Am. J. Psychiatry	1997;154:1051-1056	SMRI 35 A;26m;18y 36 C;20m;20.2y CC (3 subregions)	↓ body and posterior CC
	Egaas et al.	Arch. Neurol.	1995;52:794-801	SMRI 51 A;45m;15.5y 51 C;45m;15.5y CC (5 subregions)	↓ overall CC, especially posterior
	Saitoh et al.	Neurology	1995;45:317-324	SMRI 33 A;30m;13.8y 23 C;19m;13.3y CC (5 subregions)	↓ posterior CC
	Gaffney et al.	Br. J. Psychiatry	1987;151:831-833	SMRI 13 A;10m;11.3y 35 C;21m;12y CC	↓ CC, although not significant On 0.5 T
MTL	Herbert et al.	Brain	2003;126:1182-1192	SMRI 15 A157m;7-11y 17 C; 17m;7-11y TBV, GM, WM, CB, basal ganglia, thalamus, AMY, HIP	↓ AMY-HIP after correction TBV
	Rojas et al.	Neurosci. Lett.	2002;328:237-240	SMRI 15 A;13m;29.9y 15 C;13m;30.4y GM in PT and HG	No difference in HG; ↓ PT in left hemisphere
	Sparks et al.	Neurology	2002;59:184-192	SMRI 45 ASD;38m;4y 14 DD;6m;4y 26 C;18m;4y TBV, CB, AMY, HIP	↑ AMY and HIP, proportional to ↑ TBV
	Pierce et al.	Brain	2001;124:2059-2073	SMRI 7 A;7m;29.5y 8 C;8m;28.3y FG, ITG, MTG, AMY	↓ left and right AMY
	Saitoh et al.	Brain	2001;124:1317-1324	SMRI 59 A;52m;11.2y 51 C;40m;11.4y 3 slices with AD, CAS	↓ AD
	Haznedar et al.	Am. J. Psychiatry	2000;157:1994-2001	SMRI 17 A;15m; 27.7y 17 C;15m;28.8y AMY, HIP, CG	↓ right anterior CG, specifically Brodmann's area 24'; ↑ left AMY in AS

	Howard et al.	NeuroReport	2000;11:2931-2935	SMRI 10A;10m;16-40y 10 C;10m;? AMY, HIP, PHG, TL	↑ AMY bilaterally; trend towards ↓ HIP and PHG
	Abell et al.	NeuroReport	1999;10:1647-1651	SMRI/VBM 15 A;12m;28.8y 15 C;12m;25.3y VBM of whole brain	↓ GM right paracingulate sulcus, left IFG; ↑ GM AMY/peri-AMY cortex, MTG, ITG
	Aylward et al.	Neurology	1999;53:2145-2150	SMRI 14 A;14m;20.5y 14 C;14m;20.3y AMY, HIP	↓ AMY bilaterally; ↓ HIP (after correction TBV)
	Piven et al.	J. Aut. Dev. Disord.	1998;28:105-110	SMRI 35 A;26m;18y 36 C;20m;20.2y HIP	No differences
	Haznedar et al.	Am. J. Psychiatry	1997;154:1047-1050	SMRI 7 A;5m;24.3y 7 C;5m;26.4y anterior CG	↓ right Brodmann's area 24'; ↑ Brodmann's area 25
	Saitoh et al.	Neurology	1995;45:317-324	SMRI 33 A;30m;13.8y 23 C;19m;13.3y cross-sectional posterior HIP	No differences

Abbreviations in alphabetical order. A: autistic individual; AD: area dentata (dentate gyrus + CA4); AMY: amygdala; AS: Asperger syndrome; ASD: autism spectrum disorder; C: control subject; CAS: CA1-CA3 + subiculum; CB: cerebellum; CC*: corpus callosum, the only measurement that is not volumetric, but a midsagittal area measurement; CG: cingulate gyrus; CSF: cerebro-spinal fluid; DD: developmental delay; DLD: developmental language disorder; FR: frontal; GM: gray matter; HC: head circumference; HG: Heschl's gyrus; HIP: hippocampus; IC: intracranial volume; IFG: inferior frontal gyrus; ITG: inferior temporal gyrus; MR: mental retardation; MTG: medial temporal gyrus; MTL: medial temporal lobe; nm: not mentioned; NS: not significant; OC: occipital; PA: parietal; PHG: parahippocampal gyrus; PT: planum temporale; SMRI: structural MRI; TBV: total brain volume; TE: temporal; TL: temporal lobes; VBM: voxel-based morphometry; vent: ventricles; WM: white matter; ^: mental age;

Diffusion Tensor Imaging (DTI)

Diffusion Tensor Imaging (DTI) is a relatively new, non-invasive MRI technique which enables the investigation of the orientation of brain pathways in vivo [Foong et al. 2002]. In axons, water diffusion is impeded by myelin sheaths and cell walls. As a result, water movement along the axis of an axon is much faster than in the perpendicular direction [Cercignani and Horsfield 2001]. Thus, the direction of the fastest diffusion would indicate the overall orientation of the fibers [Le Bihan et al. 2001]. Visualization of the movement of water molecules allows visualization of the structure and direction of axons within a DTI brain image. However, a major technical limitation is the relatively coarse spatial resolution [Lim and Helpern 2002].

To date, no DTI studies have been performed in autism, but it is very likely that in the next few years, DTI studies will be performed in autism, which is a great gain in the research field, as it yields the possibility to visualize the reported white matter abnormalities in more detail.

Conclusions of structural imaging studies

In this review we have attempted to provide an extensive overview of the available structural neuroimaging literature in autism. Despite the large number of structural imaging studies in autism, results are rather inconclusive. This may be due, on the one hand, to lack of statistical power in the face of the heterogeneity of the disorder itself, and, on the other hand, to the failure to control for potential confounding variables, such as age, sex, IQ, socioeconomic status, and medication status. However, when considering those studies with large sample sizes and adequate matching, several statements can be made: (1) One of the most reliable findings about brains in autism is their increased brain volume [Frith 2002]. However, both the beginning and the possible ending of the brain enlargement remain issues of debate. The increase is not present at birth, but is thought to emerge some time after the age of 2 [Courchesne et al. 2001;Lainhart et al. 1997;Stevenson et al. 1997]. Some studies suggest that brain volume enlargement is limited to (early) childhood [Aylward et al. 2002;Courchesne et al. 2001], whereas other studies show evidence of brain enlargement still present in adolescence and adulthood [Hardan et al. 2001a;Piven et al. 1995;Piven et al. 1996]. An interesting explanation was put forward by Akshoomoff et al., hypothesizing that increased brain volume in autism might be limited to higher functioning children and adults with autism [Akshoomoff et al. 2002], an explanation in accordance with the results to date. (2) Cerebellar volume is likely to be increased in autism. (3) In contrast to the increased brain and cerebellar volume, the (posterior) midsagittal corpus callosum seems to be smaller in autism. Interestingly, the corpus callosum has been reported to be smaller in individuals with ADHD as well [Giedd et al. 1994;Hynd et al. 1991;Semrud-Clikeman et al. 1994]. Given that symptoms of

inattention and hyperactivity are both frequent in autism and ADHD, one can speculate on a possible association between callosal size and these symptoms. The findings on other brainparts are less consistent. For example, it remains to be established whether brain enlargement is global or more prominent in specific cortical lobes, although posterior increase of the cerebrum has been suggested [Piven et al. 1996]. Furthermore, results are equivocal as to whether enlargement of the cerebrum is confined to the gray and or to the white matter. Concerning the subcortical structures (amygdala, hippocampus, basal ganglia, and thalamus), no definite statements can be made, as too few studies addressed these structures and the results are quite contradictory. However, some consensus seems to exist towards (4) enlargement of the amygdala.

Thus, future structural MRI studies are needed to clarify the issues of debate, mentioned above. Furthermore, characterization of the different brain phenotypes in autism can provide evidence for specific brain-behavior relationships - as was done in the study of caudate volume and stereotyped behavior [Sears et al. 1999]- as well as provide information about the underlying mechanisms causing these phenotypes. Combination studies of both structural and functional imaging techniques may further add in elucidating these brain-behavior correlations. With new approaches, such as diffusion tensor imaging (DTI), brain connectivity can be shown by means of water diffusion through myelin tracts in the brain. This will provide new information about white matter connections and the integrity of communication pathways. Finally, the development of animal models of autism will be crucial for validating genetic and nongenetic explanations, as well as testing the direct impact of certain biological and behavioral treatments.

In conclusion, with future large-scale longitudinal studies, starting at very young ages, including homogeneous groups of patients and extensively matched control groups, and making use of (combinations of) newer and more sophisticated techniques, structural imaging studies hold a great promise to further elucidate the enigma of autism.

Note added after publication

Since this review was accepted for publication in the Journal of Neural Transmission in September 2003, neuroimaging studies published afterwards are not discussed. For completeness, we added neuroimaging articles, appearing before the end of 2004, in the following paragraph.

Total brain volume

In 2003, Levitt and colleagues were the first to map cortical sulcal patterns in autism [Levitt et al. 2003]. Using MRI scans, detailed maps of 22 major sulci were created of 21 high-functioning autistic and 20 control children and adolescents. An anterior and superior shifting of the superior frontal sulci bilaterally and an anterior shifting of the right Sylvian fissure, the superior temporal sulcus and the left inferior frontal sulcus were found in the autistic group relative to the control group. These brain areas are known to be involved in working memory, emotion processing, language, and eye gaze, all functions reported be impaired in autism. As studies of normal development suggest a posterior shifting of these sulci with age [Blanton et al. 2001], the present findings were suggested to indicate delayed maturation in these brain regions, as was suggested previously [Zilbovicius et al. 1995]. In 2004, several structural imaging studies appeared, using different techniques, investigating different subject samples and posing a variety of research questions. Two groups performed a voxel-based morphometry (VBM) study [Boddaert et al. 2004;Waiter et al. 2004]. Boddaert and colleagues investigated 21 (very) low-functioning autistic children and 12 control children of normal intelligence and found a bilaterally decrease in gray matter concentration in the superior temporal sulcus (STS) and a white matter concentration decrease in the right temporal pole and left cerebellar hemisphere in autistic children compared to control children [Boddaert et al. 2004]. The authors stated that, as the STS is increasingly recognized as a key component of the 'social brain' [Allison et al. 2000], it was likely to be abnormal in autism. In the second VBM study, the brains of 16 high-functioning autistic and 16 age-, sex-, and IQ-matched control children and adolescents were investigated [Waiter et al. 2004]. Total gray matter was found to be increased in the autism group, with local volume increases in the right fusiform gyrus, the right temporo-occipital region and the left frontal pole. A local gray matter decrease was found in the right thalamus. The decrease in global white matter did not reach significance. The increase in gray matter volume was suggested to reflect failure of apoptosis. Although both VBM studies did report abnormalities in brain areas recognized for their role in social cognition, Boddaert and colleagues reported decreases in gray matter concentrations [Boddaert et al. 2004], whereas Waiter and colleagues found an increase in gray matter in these areas [Waiter et al. 2004]. The largest difference between the two studies being the inclusion of only high-functioning subjects [Waiter et al. 2004] or the inclusion of (very) low-functioning subjects as well [Boddaert et al. 2004], this IQ difference could have contributed to the disparate findings. Apart from the 'gray matter' findings, two studies investigated specifically the white matter in autism, one using 'normal' structural MRI scans [Herbert et al. 2004] and one using the newer DTI technique [Barnea-Goraly et al. 2004]. While using diffusion tensor imaging (DTI), which enables the inves-

tigation of the orientation of brain pathways in vivo [Foong et al. 2002], seven male autistic and nine age-, gender-, and IQ-matched control children and adolescents were investigated [Barnea-Goraly et al. 2004]. Reduced fractional anisotropy (FA) values were found in the white matter adjacent to the ventromedial prefrontal cortices and in the anterior cingulate gyri as well as in the temporoparietal junctions. Additional clusters of reduced FA values were seen adjacent to the superior temporal sulcus bilaterally, in the temporal lobes approaching the amygdala bilaterally, in occipitotemporal tracts, and in the genu and body of the corpus callosum. The authors suggested that disruption of white matter tracts between these regions, implicated in social functioning, might contribute to impaired social cognition in autism. The other 'white-matter' study used a white matter parcelation technique, dividing the white matter in an outer zone –containing the radiate compartment- and an inner zone –containing sagittal and bridging system compartments [Herbert et al. 2004]. Forty-one boys (13 autistic, 14 with developmental language disorder (DLD), and 14 controls) and 22 girls (no autistic, 7 DLD, and 15 controls) with normal IQs were investigated. Enlargement of the outer, but not the inner, zone was found both in autistic and in DLD children compared to control children. In addition, both the autistic and the DLD group showed greater volume increases in later (postnatal) or longer-myelinating regions [Kinney et al. 1988]. This enlargement being in striking consistency with reports of postnatal head circumference increases in autism [Courchesne et al. 2003;Lainhart et al. 1997], an ongoing postnatal process was suggested to take place in autism. Although this study suggests that the white matter abnormalities in high-functioning autism and DLD are largely consistent, it has been suggested previously that the brain abnormalities of high- and low-functioning autistic subjects might differ [Akshoomoff et al. 2002]. Therefore, Lotspeich and colleagues investigated whether subjects with low-functioning autism (LFA), with high-functioning autism (HFA), and with Asperger's syndrome (AS) differed from one-another and from control subjects on a neuroanatomical basis [Lotspeich et al. 2004]. Cerebral tissues were investigated in 13 LFA, 18 HFA, 21 AS, and 24 age-matched control male children and adolescents. Increased cerebral gray matter was found in LFA and HFA compared to controls, whereas the AS group showed intermediate volumes, nonsignificantly different from either the HFA or the control group. In addition, a negative correlation was found between cerebral gray matter volume and performance IQ in the HFA, but not in the AS group, whereas a positive correlation was found between cerebral white matter volume and performance IQ in the AS, but not in the HFA group. It was suggested that, looking at the brain abnormalities, AS is on the mild end of the autism spectrum, although the different brain-IQ correlations between HFA and AS might indicate that these conditions are neurodevelopmentally different. Finally, Kates and colleagues were the first to investigate the genetic basis of

structural brain abnormalities in autism by investigating monozygotic twin pairs (dis)cordant for autism [Kates et al. 2004]. MRI scans were made of sixteen monozygotic twin pairs (seven concordant and nine discordant for autism, all between 5 and 14 years of age) and 16 singleton comparisons. Both concordant and discordant twin pairs exhibited concordance in cerebral gray and white matter volumes. However, only the clinically concordant pairs showed concordance in cerebellar gray and white matter volume. Thus, cerebral volumes, both gray and white matter, seemed to be largely genetically determined, whereas cerebellar volumes may be mediated by nongenetic factors. Furthermore, compared to the controls, the discordant twins did not show differences in total brain or cerebral gray matter volume. However, the unaffected twins showed a decrease of 7.5% and the affected discordant twins a decrease of even 11.5% in white matter volume.

Corpus callosum

As there is little understanding about the link between the functional deficit and the underlying abnormal anatomy in autism, Chung and colleagues performed a 2D-VBM study, investigating white matter concentrations of the corpus callosum in 16 high-functioning autistic and 12 control adolescents and young adults [Chung et al. 2004]. Compared to the control group, the autism group showed less white matter concentration in the genu, the rostrum, and the splenium –but not midbody- of the corpus callosum. These results were suggested to implicate impaired interhemispheric connectivity in frontal, temporal, and occipital –but not parietal- regions. Furthermore, as a significant 2.5%/year increase was found in the genu of the autistic group, the smaller callosal size in the autism group was hypothesized to result from hypoplasia rather than atrophy. Although decreases in callosal size have been frequently reported (see review), Herbert and colleagues did not find callosal size differences neither between 13 high-functioning autistic boys and 21 children with developmental language disorder (including 14 boys), nor between these 13 high-functioning autistic boys and 14 control boys [Herbert et al. 2004].

Medial temporal lobe structures

Using single-case VBM, Salmond and colleagues investigated the hippocampus, amygdala, orbitofrontal cortex (OFC), superior temporal gyrus (STG), and cerebellum in 14 high-functioning autistic and 18 control children and adolescents [Salmond et al. 2003]. Hippocampus and amygdala showed abnormalities in 7 autistic subjects, OFC in 13, STG in 10, and cerebellum in 11. Unfortunately, 'abnormalities' were not defined, so it remains unclear whether they implied increased or decreased gray matter concentrations. It was concluded that, as no neural area was found to be significantly abnormal in all of the autistic children, the autistic phenotype might reflect abnormalities within multiple systems. Indeed, some form of temporal lobe abnormality has

been suspect in autism since the early 70s [Hauser et al. 1975]. Therefore, Bigler and colleagues investigated temporal lobe structures in autism in more detail, taking into account potential confounding factors, such as brain size and IQ [Bigler et al. 2003]. Apart from the amygdala and the hippocampus, the temporal stem and 5 major temporal gyri (superior, middle, inferior, fusiform, and parahippocampal) were investigated in 38 autistic males (12 macrocephalic and 26 normocephalic), 27 control males (8 macrocephalic and 19 normocephalic), and 17 males with reading disorder. No volumetric temporal lobe abnormalities were observed once head size and IQ were controlled for, although a nonsignificant decrease in white matter volume in the region of the temporal stem was found in the autistic group. Thus, it was suggested that temporal lobe abnormalities that might be associated with autism were likely to be more related to functional organization within the temporal lobe than to any gross volumetric difference. Finally, both the hippocampus and the amygdala were examined in four diagnostic groups of male children and adolescents: autism with mental retardation (n=18), autism without mental retardation (n=21), Asperger's syndrome (n=24), and typically developing children (n=22) [Schumann et al. 2004]. All groups were split into a child group (7.5-12.5 years of age) and a adolescent group (12.5-18.5 years of age). Both autistic children with and without mental retardation showed larger left and right amygdala volume, contrary to autistic adolescents, showing no differences in amygdala volumes compared to controls. As the amygdala in typically developing children increased with age, it was hypothesized that in autistic children this age-related increase in amygdala volume failed to take place, resulting in normalizing of amygdala volumes by adolescence. Concerning the hippocampus, all subjects with autism showed larger right hippocampal volume, whereas only those autistic subjects, both children and adolescents, without mental retardation also showed increased left hippocampal volume. Thus, the increase in (right) hippocampal volume seemed to be persistent. It was suggested that the volume of the hippocampus –correlated with spatial memory function- might show a use-dependent enlargement, as was found in London taxi-cab drivers [Maguire et al. 2000;Maguire et al. 2003]. Indeed, enhanced spatial or episodic memory function has recently been reported in autism [Caron et al. 2004].

In conclusion, these additional studies did not resolve the problem of contradictory neuroimaging findings in autism. Thus, the conclusion, earlier drawn remains valid: large-scale longitudinal studies, starting at very young ages and including homogeneous groups of patients and extensively matched control groups are needed in order to be able to further elucidate the enigma of autism.

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Chapter 3

Increased gray matter volume in medication-naive high-functioning children with autism spectrum disorder

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Abstract

Background: To establish whether high-functioning children with autism spectrum disorder (ASD) have enlarged brains in later childhood, and if so, whether this enlargement is confined to the gray and/or to the white matter and whether it is global or more prominent in specific brain regions. **Methods**: Brain MRI scans were acquired from 21 medication-naive, high-functioning children with ASD between 7 and 15 years of age and 21 comparison subjects matched for gender, age, IQ, height, weight, handedness, and parental education, but not pubertal status. **Results:** Patients showed a significant increase of 6% in intracranium, total brain, cerebral gray matter, cerebellum, and of more than 40% in lateral and third ventricles compared to controls. The cortical gray matter volume was evenly affected in all lobes. After correction for brain volume, ventricular volumes remained significantly larger in patients. **Conclusions**: High-functioning children with ASD showed a global increase in gray –but not white- matter and cerebellar volume, proportional to the increase in brain volume, and a disproportional increase in ventricular volumes, still present after correction for brain volume. Advanced pubertal development in the patients compared to the age-matched controls may have contributed to the findings reported in the present study.

Introduction

Autism is a neurodevelopmental disorder of unknown origin defined by the presence of marked social deficits, specific language abnormalities, and stereotyped, repetitive behaviors [American Psychiatric Association 1994]. An accumulating body of evidence suggests that in a subset of autistic children, head and brain size are increased. Kanner, the first to report on autism, noticed the presence of enlarged heads in some children with autism [Kanner 1943]. Several subsequent studies have replicated this finding (see e.g. [Aylward et al. 2002;Lainhart et al. 1997]). Consistent with these clinical findings, neuropathological studies have reported increased brain weight in some, but not all children with autism [Bailey et al. 1998;Casanova et al. 2002a;Courchesne et al. 1999;Kemper and Bauman 1998]. Likewise, neuroimaging studies have reported increased brain size in autistic children, but not consistently [Aylward et al. 2002;Carper et al. 2002;Courchesne et al. 2001;Filipek et al. 1992;Herbert et al. 2003;Sparks et al. 2002].

To date, no specific characteristics have been found that discriminate between subjects with and without enlarged brains, but IQ and age seem to be factors of importance. With respect to IQ, it has been suggested that abnormal brain development is a characteristic feature of autism regardless of IQ [Aylward et al. 2002]. However several studies have revealed a significant correlation between brain volumes and intelligence measures in normal controls (see e.g. [Posthuma et al. 2002;Thompson et al. 2001]). Furthermore, the mean head circumference of mentally retarded individuals in general is two or more standard deviations below the means of normal individuals [Mosier, Jr. Et al. 1965;Nellhaus 1968]. Hence, increased brain volume associated with autism may be cancelled out by decreased brain volume associated with mental retardation, at least when low-functioning autistic children are compared to children with normal intelligence. Indeed, enlarged brain volumes were found in studies of school aged children with high-functioning autism, compared to control children [Aylward et al. 2002;Filipek et al. 1992], although not always significantly [Herbert et al. 2003], but not in studies including low-functioning autistic children [Courchesne et al. 2001]. So, when investigating the relation between autism and brain volume, it is of importance to include only high-functioning autistic children.

Concerning the age factor, results have consistently shown brain enlargement in very young autistic children, either in whole brain volume [Courchesne et al. 2001;Sparks et al. 2002], or in specific cortical volumes [Carper et al. 2002], whereas studies in older autistic children show either no brain enlargement [Carper et al. 2002;Courchesne et al. 2001], or only enlargement up to the age of 12 [Aylward et al. 2002] when compared to control children. At the same time, several studies show evidence of brain enlargement in autism still present in adulthood [Hardan et al. 2001;Piven et al. 1995;Piven et al. 1996]. Thus, it is

unclear whether brain enlargement in autism is a phenomenon restricted to early childhood or whether it remains present in older children. A related question is whether this enlargement is global or whether it is confined to specific brain parts. Increased frontal, parietal, and temporal, but not occipital, regions were found in autistic children aged 2-3, but not in older children (aged 4-16) [Carper et al. 2002]. Increased cerebellar volume has consistently been reported in autistic children [Courchesne et al. 2001;Filipek et al. 1992;Herbert et al. 2003;Sparks et al. 2002], whereas ventricular volume has been found to be either increased [Gaffney et al. 1989] or comparable for autistic children and normal control children [Filipek et al. 1992]. Recently, technical developments in neuroimaging allow the distinction between cortical gray and white matter. The few studies to date that have investigated cerebral gray and white matter separately in autistic children, yielding contradictory results. Increased gray and white matter volume was reported in autistic children aged 2-3 years, but not in autistic children aged 4-16 [Courchesne et al. 2001]. Recently, increased white matter volume was reported for high-functioning autistic children, aged 7-11 years, whereas a decrease in cerebral gray matter volume was found [Herbert et al. 2003]. In studies on gray and white matter, the age factor might be of special importance, since in normal development this proportion changes with age [Giedd et al. 1999]. Careful matching on age of autistic and control children is therefore needed.

It is known that, besides IQ and age, several other factors, such as gender, handedness, length, weight, socioeconomic status, and the use of (neuroleptic) medication are correlated to the size of various brain structures. Some of these factors seem to be especially important in studies on brain size in autism. While there are no reports on systematic differences between autistic subjects and normal controls with respect to height, weight, and socioeconomic status, autism is known to be associated with a 4:1 male/female ratio, with a higher prevalence of left or ambiguous handedness [Hauck and Dewey 2001], and with the use of neuroleptics. With respect to medication, it is known from studies on subjects with schizophrenia that neuroleptics, which are also regularly prescribed to autistic subjects, affect the size of several brain structures [Chakos et al. 1994;Scheepers et al. 2001].

Therefore, matching on IQ, age, gender, handedness, and inclusion of only medication-naive patients, is especially important when comparing children with ASD with typically developing children, and the present study is the first to do so. In addition, we determined the Tanner score of all our subjects, indicating the level of sexual development. The reason is that the gender differences in brain size are likely to be related to sex-hormonal levels, and these have been shown to be abnormal in autism [Tordjman et al. 1997].

We compared brain scans of a group of 21 medication-naive high-functioning children with ASD, between 7 and 15 years of age, with those of 21 typically

developing children. The aim was to determine (1) whether brain enlargement is present in children with ASD up to age 15, and if so, (2) whether this enlargement is confined to the gray and/or to the white matter and/or to the cerebrospinal fluid, and (3) whether enlargement is global or more prominent in specific cortical volumes.

Methods

Subjects

Twenty-one medication-naive, high-functioning patients fulfilling the DSM-IV criteria of autism [American Psychiatric Association 1994] were recruited through the National Autism Society. To confirm clinical diagnosis, parental informants for all patients were interviewed with the ADI-R [Lord et al. 1994]. All patients fulfilled the ADI–R algorithm for both the social behavior and the communication subscores. Four patients scored just below the threshold for the ritualistic-repetitive behavior subscore. Twenty-one normally developing control children were recruited through schools in the area. For each control subject a parent was asked to participate in a semi-structured interview session with a trained rater to confirm absence of any psychiatric diagnosis (Diagnostic Interview Schedule for Children (DISC-P); [Costello et al. 1985]). All subjects participated after parental written consent was obtained. Subjects with major physical or neurological illness, such as migraine, epilepsy, head trauma in the past or full IQ below 80 were excluded. All subjects were asked to participate in a 35-minute MRI scanning session and a neuropsychological assessment in order to estimate full-scale IQ (Wechsler Intelligence Scale for Children - Revised (WISC-R); [Wechsler 1974]). All subjects were males and between 7 and 15 years of age. The procedure was approved at the institutional review board of the University Medical Center in Utrecht, the Netherlands. Patients and healthy comparisons were matched for gender, age, total IQ, verbal IQ, performance IQ, height, weight, handedness, and for the socioeconomic status of their parents expressed as the highest completed level of education by either parents. However, the difference in pubertal status between patients and controls was significant (see table 1 for demographics).

Brain MRI scans from all subjects were evaluated by two independent clinical neuroradiologists. No gross abnormalities were reported in any of the subjects.

Table 1: Demographic data

	Patients	Comparison subjects
Participants (all male), no	21	21
Age, mean ± SD (range), y	11.12 ± 2.18 (6.9 – 14.6)	10.37 ± 1.84 (7.3 – 14.4)
Total IQ, mean ± SD (range)	106.52 ± 13.68 (80 – 138)	102.52 ± 14.58 (80 – 151)
Verbal IQ, mean ± SD (range)	108.33 ± 17.54 (70 –131)	100.86 ± 15.81 (76 – 144)
Performance IQ, mean ± SD (range)	103.43 ± 16.81 (73 – 141)	103.62 ± 14.54 (73 – 138)
Height, mean ± SD, cm #	149.19 ± 16.17	145.83 ± 15.88
Weight, mean ± SD, kg #	38.81 ± 11.79	38.61 ± 10.11
Handedness (right/left), no	20/1	19/2
Parental education, mean ± SD, y ǂ	14.10 ± 2.45	12.84 ± 2.63
Tanner A, mean ± SD ǂǂ	2.43 ± 1.33	1.55 ± 0.94 *
Tanner B, mean ± SD ǂǂ	1.48 ± 0.81	0.25 ±0.55 **
ADI: social deficits	16.38 ± 4.61	
ADI: abnormalities in communication	13.00 ± 4.59	
ADI: ritualistic-repetitive behavior	4.14 ± 2.31	

Information was unavailable for three comparison subjects

ǂ Information was unavailable for two comparison subjects

ǂǂ Information was unavailable for one comparison subject

* $|t| = 2.43$, $p = 0.020$

** $|t| = 5.62$, $p < 0.0005$

ADI: Autism Diagnostic Interview

MRI acquisition and procedures

Magnetic resonance images were acquired on a Philips Gyroscan (Philips Medical Systems, Best, the Netherlands) operating at 1.5 T. For volumetric measurements T1-weighted 3D fast field echo scans with 130 to 150 1.5 mm contiguous coronal slices of the whole head (TE 4.6 ms, TR 30 ms, flip angle 30º, field of view (FOV) 256 mm, in plane voxel size 1 mm x 1 mm) and T2-weighted dual echo turbo spin echo scans with 65 to 75 3.0 mm contiguous coronal slices (TE1 14 ms, TE2 80 ms, TR 6350 ms, flip angle 90º, FOV 256 mm, in plane voxel size 1 mm x 1 mm) were acquired. In addition, T2-weighted dual echo turbo spin echo scans with 17 axial 5 mm slices and a 1.2 mm gap (TE1 9 ms, TE2 100 ms, flip angle 90º, FOV 250 mm, in plane voxel size 0.98 mm x 0.98 mm) were acquired for clinical neurodiagnostic evaluation. All processing was performed on the neuroimaging computer network of the Department of Psychiatry including workstations (Unix 9000; Hewlett Packard, Palo Alto, CA),

a compute server, and Pentium III personal computers. MRI scans were coded to ensure masking for subject identity and diagnosis. Analysis started with placing the MR scans in a Talairach [Talairach and Tournoux 1988] frame without scaling (AC-PC alignment), followed by correction for inhomogeneities in the magnetic field [Sled et al. 1998]. Quantitative assessments of the intracranial, total brain, cerebral gray and white matter (total brain excluding cerebellum and stem), lateral and third ventricles, and peripheral cerebrospinal fluid (CSF) volumes were performed based on histogram analyses and series of mathematical morphological operators to connect all voxels of interest, validated previously [Schnack et al. 2001a]. A plane through the fourth ventricle and the aqueduct limited the cerebellum. In lateral ventricle segmentation automatic decision rules bridged connections not detectable and prevented 'leaking' into cisterns [Schnack et al. 2001b]. Coronal slices clearly showing the anterior and posterior commissures limited the third ventricle; the upper boundary was a plane through the plexus choroideus ventriculi tertii perpendicular to the midsagittal slice. All images were checked after the measurements and corrected manually if necessary using Analyze TM [Robb 1995] and DISPLAY (Mc Conell Brain Imaging Centre, Montréal Neurological Institute, McGill University). Ten brains were randomly selected and analyzed by two independent raters in order to estimate interrater reliability. Intraclass Correlation Coefficients were 0.998 for intracranial volume, 0.998 for total brain volume, 0.952 for gray matter volume, 0.958 for white matter volume, 0.984 for cerebellum volume, 0.999 for lateral ventricle volume, and 0.850 for third ventricle volume. Frontal, parietal, temporal, and occipital lobes were manually demarcated on a brain image that served as a model. The model brain was selected earlier among 200 brain images of healthy subjects between 16-70 years of age [Mandl et al. 1999]. The frontal lobes were limited by the frontal pole, lateral fissure, and interhemispheric, circular insular, central, olfactory, and cingulate sulci. The parietal lobes were limited by the central, interhemispheric, circular insular, subparietal, and cingulate sulci, lateral ventricles, and lateral, and parieto-occipital fissures. The temporal lobes were limited by the lateral, circular insular, anterior calcarine, and interhemispheric sulci, amygdala-hippocampal complex, lateral ventricles, and temporo-occipital notch. The occipital lobes were limited by the interhemispheric sulci, parieto-occipital fissure, and temporo-occipital notch [Palmen et al. in press]. Brain images were registered to the model brain through the ANIMAL algorithm [Collins et al. 1996] to remove global differences in size and shape of the individual brains. The inverse of the transformation process registered the manual segmentations of the model brain to all subjects' brain images. The gray and white matter segments from the individual brain images were used to divide model-based lobar segmentations into gray and white matter.

Statistical analyses

All clinical data and brain volume measures were found to be normally distributed.

One control child had an extremely large lateral ventricle volume. Therefore, data were analyzed both with and without this subject.

To examine whether brain volumes differed between the patient and the control group, multiple analyses of variance (ANOVAs) were done with intracranial, total brain, frontal, parietal, temporal, and occipital lobe, gray and white matter of the cerebrum (total brain, excluding cerebellum and brainstem), gray and white matter of the four cortical lobes, cerebellum, and lateral and third ventricular volume as dependent variables and group (ASD versus control children) as independent variable.

Using ANCOVAs, analyses were repeated both with intracranial volume and total brain volume as covariates to examine whether volumetric differences in brain volumes could be explained by differences in intracranial volume between patients and control children and to examine whether the brainpart volumes were (dis)proportionately increased (or decreased). As there was a significant difference in Tanner stages between patients and controls, Tanner stages were included as covariates in the analyses.

SPSS 9.0 statistical package for Windows (SPSS Inc, Chicago, III) was used for these analyses, with a 2-tailed alpha level of 0.05. We did not correct for multiple significance tests.

Results

Demographic and matching variables revealed no significant differences between the two groups, except pubertal status. Children with ASD scored significantly higher compared to normally developing children ($t=2.43$, $p=0.020$ and $t=5.62$, $p<0.0005$, for Tanner A and Tanner B, respectively) (see table 1).

For mean (sd) values of the brain volumes see table 2. Intracranial, total brain, frontal gray, parietal gray, temporal gray, cerebellum and lateral and third ventricular volumes were significantly larger in patients compared to control children. More than 80% of the children with ASD exceeded the mean total brain volume of the control group, whereas only 19% of the controls exceeded

Table 2: Absolute brain volumes (ml) of autistic and healthy comparison subjects

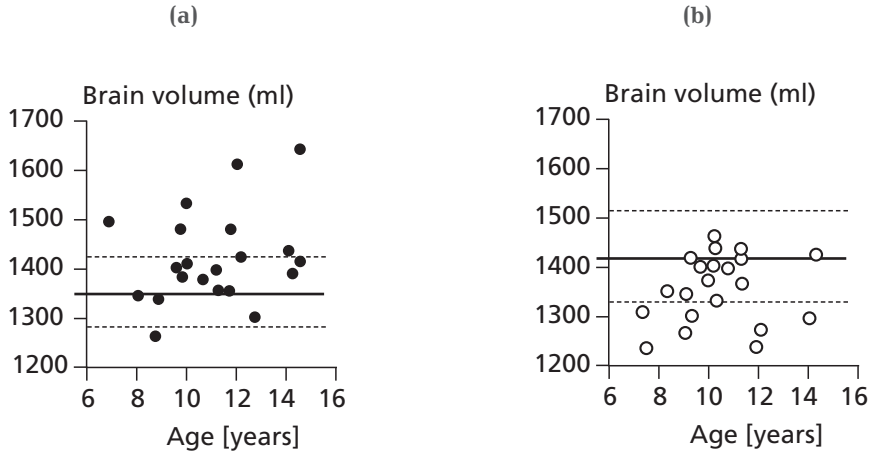
	Patients (n=21) Mean ± SD	Comparison subjects (n=21) Mean ± SD	F dí=1,40	p
Intracranium	1542.10 ±103.02	1475.17 ±69.45	6.09	0.018*
Total brain	1422.79. ±92.62	1357.85 ±70.02	6.57	0.014*
Cerebral gray matter	787.43 ±67.45	743.45 ±49.11	5.84	0.020*
Cerebral white matter	465.94 ±34.51	452.83 ±30.79	1.69	0.201
Frontal gray matter	267.93 ±23.09	253.16 ±19.70	4.98	0.031*
Frontal white matter	169.92 ±12.61	162.50 ±11.97	3.83	0.057
Parietal gray matter	146.24 ±13.98	136.84 ±10.09	6.25	0.017*
Parietal white matter	82.71 ±7.06	78.66 ±6.63	3.68	0.062
Temporal gray matter	164.94 ±14.68	156.35 ±8.78	5.29	0.027*
Temporal white matter	63.07 ±5.73	64.16 ±6.22	0.34	0.561
Occipital gray matter	71.30 ±7.76	67.33 ±6.36	3.29	0.077
Occipital white matter	46.68 ±6.32	45.85 ±4.83	0.23	0.632
Cerebellum	155.17±11.76	147.35 ±11.86	4.60	0.038*
Lateral ventricles	12.41 ±5.39	8.56 ±5.82	4.94	0.032*
Third ventricle	0.66 ±0.21	0.45 ±0.18	13.10	0.001**

* Indicates the $p<0.05$ and ** the $p < 0.01$ significance level of comparisons between patients and healthy comparison subjects by analysis of variance

the mean total brain volume of the ASD group (figure. 1).

Figure 1:

Total brain volume in (a) patients and (b) normal control children. Thick black line represents the mean brain volume, whereas the dotted lines represent the standard deviations of the controls (figure a) and of the patients (figure b). As shown, more than 80% (17/21) of the patients had whole brain volumes larger than normal mean. In contrast, only 19% (4/21) of normal children exceeded the patient mean.



After correction for intracranial volume, lateral –without the extreme value- ($F=7.55$, $df=1,38$, $p= 0.009$) and third ($F=7.91$, $df=1,39$, $p=0.008$) ventricular volume remained significantly larger in the ASD group. Using total brain volume as covariate did not alter the results significantly.

After inclusion of Tanner stages as covariates, differences in brain volumes between patients and controls no longer reached significance.

Exclusion of the four children with ASD who scored just below the threshold for the ADI subscore "ritualistic and repetitive behavior" did not alter the results.

Discussion

This cross-sectional study compared brain morphology in 21 medication-naive high-functioning children with ASD between 7 and 15 years of age and 21 control children, matched on gender, age, IQ, length, weight, handedness and parental education, but not on pubertal status. Its main finding is a significant

global increase of 5%-6% in intracranial, total brain, cerebral, frontal, parietal, and temporal gray matter, and cerebellar volume, and an increase of more than 40% in lateral and third ventricular volume in children with ASD compared to typically developing children. After correction for intracranial or total brain volume, lateral and third ventricular volume remained significantly enlarged in the ASD group compared to the control group. Cerebral white matter volume did not differ between the two groups.

The increase in total brain (and cerebellum) volume in children with ASD is consistent with earlier reports [Aylward et al. 2002;Filipek et al. 1992;Sparks et al. 2002], although younger age ranges were included in these studies (8-12 years, 6-10 years and 3-4 years of age, respectively). Another study found cerebral and cerebellar enlargement, but only in children aged 2-3 years (not in children between 4-16 years of age) [Courchesne et al. 2001]. These different results may be related to differences in characteristics between the patient group of the present study and the patient groups included in other studies. As was put forward earlier, increased brain volume may be found only in high-functioning autistic children [Akshoomoff et al. 2002]. Indeed, studies reporting increased brain volume in school aged autistic children, included high-functioning autistic subjects [Aylward et al. 2002;Filipek et al. 1992], although in one study, the difference in brain volume did not reach significance ($p=0.077$) [Herbert et al. 2003]. In addition, reports of increased brain volume in adolescents and adults with autism have typically included high-functioning autistic and IQ-matched control subjects [Hardan et al. 2001;Piven et al. 1995;Piven et al. 1996]; but see [McAlonan et al. 2002].

The increase in cerebellar volume in children with ASD is consistent with some earlier studies [Filipek et al. 1992;Herbert et al. 2003;Sparks et al. 2002], although one study did not find cerebellar enlargement in older autistic children [Courchesne et al. 2001].

Interestingly, the increase in both lateral and third ventricular volume was disproportionally large and still present after correction for total brain volume. This finding has not been reported before, probably as other studies have typically not corrected for brain volume [Courchesne et al. 1987;Filipek et al. 1992;Gaffney et al. 1989;Howard et al. 2000;Piven et al. 1995;Townsend et al. 2001]. However, percent brain enlargement was 1.3-6.7% in those studies [Howard et al. 2000;Piven et al. 1995;Townsend et al. 2001], whereas ventricular enlargement was 28.9-153%. Thus, it seems that the results from those studies may indicate a disproportional ventricular enlargement as well. In addition, in a follow-up study of patients with childhood-onset schizophrenia it was found that those patients who had early transient autistic features were the ones with the greatest pubertal ventricular enlargement [Rapoport et al. 1997].

The present study is the first to report on cerebral and lobar gray and white matter abnormalities in high-functioning children with ASD. A significant

increase of approximately 6% in frontal, parietal, and temporal gray - but not white- matter volume was found, an increase that was roughly proportional to the increase in brain volume. The increase in occipital gray matter volume was also approximately 6%, although this did not reach significance, probably due to the smaller occipital volume. To date, only one study has investigated cerebral - but not lobar- gray and white matter in high-functioning autistic children [Herbert et al. 2003], reporting increased cerebral white –but not gray- matter volume in 17 high-functioning autistic boys, compared to 15 controls. However, no information was available on matching variables (age, IQ, handedness, medication status), which made direct comparison to the present study difficult.
To date, two studies, investigating cerebral and lobar gray and white matter, have been published, including low-functioning subjects as well [Carper et al. 2002;Courchesne et al. 2001]. An increase in both cerebral gray and white matter volume was found in autistic children aged 2 – 3 years, but not in older autistic children (4-16 years of age) [Courchesne et al. 2001]. Increases in frontal, parietal, and temporal white matter, as well as in frontal and temporal gray matter volume were found in the same patients after dividing the cerebrum into its four cortical lobes [Carper et al. 2002]. We hypothesize that the significantly increased cerebral gray matter volume in children with ASD found in the present study could have been due to the inclusion of only high-functioning patients and IQ-matched controls. Indeed, it is known from the literature that gray (and white) matter volume and density are positively correlated with IQ [Posthuma et al. 2002;Thompson et al. 2001]. Moreover, our findings of increased gray matter volume are consistent with the reported abnormally thickened cortices in autistic brains [Bailey et al. 1998]. Furthermore, a post-mortem study reported more, but smaller minicolumns in patients with autism [Casanova et al. 2002b], pointing towards an increase in cortical surface area. The absence of white matter findings in our study is also in agreement with post-mortem research, finding no myelination abnormalities in autistic children [Bauman and Kemper 1994;Kemper and Bauman 1998]. However, none of the postmortem studies quantified volumetric measurements.
As to the nature of the underlying pathophysiology of the brain enlargement in ASD one can only speculate. Our results suggest that the increased brain volume in ASD is due to enlargement of the gray –but not white- matter volume. Decreased elimination of neural processes –including apoptosis, axonal pruning, and dendritic degeneration-, as well as increased neurogenesis have been suggested to occur in autism [Piven et al. 1996]. Our finding of increased gray matter volume seems to be consistent with the reported increased N-acetylaspartate (NAA) in the prefrontal lobe of subjects with Asperger's syndrome [Murphy et al. 2002] as increased NAA suggests more (active) neurons.
Serotonin is known to block apoptosis [Azmitia 2001]. Thus, it is possible that the reported hyperserotonemia in autism [Veenstra-vanderweele et al. 2002]

excessively blocks apoptosis. Moreover, in Rett's syndrome, another pervasive developmental disorder, loss of serotonin produces a reduction in the thickness of gray matter, not a reduction of myelin [Azmitia 2001]. Thus, hyperserotonemia may produce increased thickness of the gray matter in autism without affecting the white matter. Regarding the enlarged ventricles, one could speculate that subcortical structures in the neighborhood of the ventricles are smaller in ASD. However, to date, two studies investigated the basal ganglia, one reporting an increase in caudate volume [Sears et al. 1999] and the other in putamen-globus pallidus volume [Herbert et al. 2003]. On the other hand, a recent study investigated the thalamus and reported decreased thalamic volume in those autistic subjects who had large brains [Tsatsanis et al. 2003]. Another possible explanation may be that the brain is enlarged more than 6% in patients younger than 7 years of age and that therefore brain volume decreases relatively more rapidly in patients compared to controls, resulting in disproportionally increased ventricular volume. Aside of this neurodegenerative explanation, birth trauma may have been responsible for the enlarged ventricles [McNeil et al. 2000]. Unfortunately we do not have birth records of the patients, so we cannot retrospectively look for possible birth trauma. Future (post-mortem) research is necessary to clarify the underlying mechanisms of increased gray matter volume and disproportional enlarged ventricles in patients found in the present study.

Finally, after covariance for Tanner stages, the differences in brain volumes between patients and controls no longer reached significance, implying that the advanced pubertal development in patients compared to controls may have contributed to the differences in brain volumes, reported in the present study. This finding of significantly higher Tanner scores in the patient group was rather unexpected, as the subjects were closely matched on age. However, precocious secondary sexual characteristics and abnormally high plasma testosterone have been reported before in autistic children [Tordjman et al. 1997]. Furthermore, the 2^{nd}: 4^{th} digit ratio, which is negatively correlated with prenatal testosterone, is lower in children with autism [Manning et al. 2001]. In addition, the postnatal peak in testosterone has a significant role in genital development and postnatal androgen suppression causes delayed onset of puberty [Main et al. 2000]. Thus, it is speculated that pre- and/or postnatal testosterone may be elevated in subjects with ASD resulting in precocious puberty. Indeed, elevated testosterone would be in accordance with enlarged brain volumes, as the male brain is larger than the female brain [Giedd et al. 1996]. Future research, measuring testosterone in very young children suspected to have ASD is necessary to elucidate the intricate finding of more advanced pubertal development in boys with ASD compared to age-matched typically developing children.

Limitations

This study was limited in several aspects. These limitations should be taken into consideration when interpreting its findings. First, its relatively small sample size. The present findings of increased gray matter volume in high-functioning patients with ASD need to be replicated in a larger sample. Second, because this study was restricted to high-functioning patients, results may not be representative of mentally retarded patients, representing about 75% of all autistic individuals. Third, although the two groups were matched on seven factors, they were not matched on pubertal status, which could have contributed to the reported increased brain volume in the ASD group. However, although both groups were closely matched on age –which is a must in structural MRI research-, and both groups showed significant positive correlations between age and pubertal status (data not shown here), it was simply not possible to match both groups on pubertal status. This finding may represent strong evidence for a direct relation between autism and higher levels of sexual development and may have direct implications for the interpretation of the consistently reported enlarged brains in autism. Fourth, the present study only measured volumes of (large parts of) the brain. It remains to be investigated whether focal, subcortical gray matter structures are abnormal in these patients.

Conclusions

In conclusion, we have found brain enlargement in medication-naive high-functioning children with ASD, compared to a control group, matched on gender, age, IQ, length, weight, handedness, and parental education, but not on pubertal status. Intracranium, total brain, cerebral, frontal, parietal, and temporal gray matter, and cerebellum volume, were all proportionally enlarged, whereas the ventricular volumes were disproportionally enlarged. Thus, medication-naive high-functioning children with ASD showed brain enlargement still present in later childhood. Advanced pubertal development in the patients compared to the age-matched controls may have contributed to the findings reported in the present study.

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Chapter 4

Larger brains in medication-naive high-functioning adolescents with Pervasive Developmental Disorder

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Abstract

Background: Are brain volumes of individuals with Pervasive Developmental Disorder still enlarged in adolescence and adulthood, and if so, is this enlargement confined to the gray and/or the white matter and is it global or more prominent in specific brain regions. **Methods:** Brain MRI scans were made of 21 adolescents with PDD and 21 closely matched controls. **Results:** All brain volumes, except the white matter, were significantly larger in patients. After correction for brain volume, ventricular volumes remained significantly larger in patients. **Conclusions:** Patients showed a proportional, global increase in gray matter and cerebellum volume, and a disproportional increase in ventricular volumes. Thus, at least in high-functioning patients with PDD, brain enlargement may still be present in adult life.

Introduction

Autism is a neurodevelopmental disorder of unknown origin defined by the presence of marked social deficits, specific language abnormalities, and stereotyped, repetitive behaviors [American Psychiatric Association 1994]. It is the most severe disorder among the Pervasive Developmental Disorders.

An accumulating body of evidence suggests that, at least in a subset of individuals with autism, head and brain size are increased. Kanner, the first to report on autism, noticed the presence of enlarged heads in some children with autism [Kanner 1943]. Several subsequent studies have replicated this finding both in children and in adults [Aylward et al. 2002;Bailey et al. 1993; Davidovitch et al. 1996;Fidler et al. 2000;Fombonne 2000;Gillberg and de Souza 2002;Lainhart et al. 1997;Miles et al. 2000;Stevenson et al. 1997; Woodhouse et al. 1996]. Likewise, neuropathological studies have reported increased brain weight in a subset of subjects with autism. Percentages of brain weight above the 98th percentile of 19% (4 out of 21) [Courchesne et al. 1999] and 50% (3 out of 6) [Bailey et al. 1998] were reported in autistic subjects. Another study reported increased brain weight in 73% (8 out of 11) of autistic children younger than 12 years of age, but not in 8 autistic subjects older than 18 years [Kemper and Bauman 1998]. In accordance with these reports, several neuroimaging studies have reported increased brain size in autism. Reports of increased brain size are unequivocal in children [Aylward et al. 2002;Carper et al. 2002;Courchesne et al. 2001;Filipek et al. 1992;Sparks et al. 2002]. In contrast, in autistic adolescents and adults, compared to control subjects, contradictory results of either increased brain volume [Hardan et al. 2001a;Hardan et al. 2001b;Piven et al. 1995;Piven et al. 1996], or no difference in brain volume [Aylward et al. 2002;Aylward et al. 1999;Carper et al. 2002;Courchesne et al. 2001;Haznedar et al. 2000;Rojas et al. 2002;Townsend et al. 2001] were found. The reported increased overall brain volume in autistic adults [Hardan et al. 2001a;Hardan et al. 2001b;Piven et al. 1995;Piven et al. 1996] has to be explained either by increased gray matter volume and/or by increased white matter volume. The few studies to date that investigated cerebral gray and white matter separately, reported no overall differences between adolescent patients and controls [Courchesne et al. 2001], or between adult patients and controls [McAlonan et al. 2002;Townsend et al. 2001], although both cerebral gray and cerebral white matter were found to be increased in autistic children aged 2 to 3 [Courchesne et al. 2001]. Thus, it remains inconclusive as to whether abnormalities in cortical gray and/or white matter volume remain present in autistic adolescents and young adults.

Furthermore, results are equivocal as to whether abnormalities are global or more prominent in some cortical volumes. In the first study measuring the volumes of all four cerebral lobes, increased parietal, occipital and temporal, but

not frontal, lobe volume was found in autistic adults [Piven et al. 1996]. Using voxelbased morphometry, gray matter density was found to be increased in the temporal lobe and cerebellum and decreased in the frontal lobe in autistic adults [Abell et al. 1999]. In contrast, one study, measuring only the frontal lobe, reported increased frontal gray matter volume in those autistic subjects who had midsagittal hypoplasia of cerebellar lobules VI and VII [Carper and Courchesne 2000]. Most recently, increased frontal, parietal and temporal white matter and increased frontal and temporal gray matter was found in autistic children aged 2-3, but not in older children [Carper et al. 2002]. Finally, in the majority of reports, cerebellum volume was found to be increased in autistic adolescents and adults [Abell et al. 1999;Hardan et al. 2001b;Piven et al. 1997], but see [Courchesne et al. 2001]. Likewise, increased ventricular volume was suggested in most studies [Courchesne et al. 1987;Gaffney et al. 1989;Howard et al. 2000;Piven et al. 1995], but see [Hardan et al. 2001a].

Thus, although brain abnormalities exist in autistic subjects, results are equivocal. Especially the contradictory findings in autistic adolescents/adults need further exploration. It is known that gender [Caviness et al. 1996;Giedd et al. 1996;Kertesz et al. 1990;Reiss et al. 1996], age [Caviness et al. 1996;Giedd et al. 1996;Holland et al. 1986;Reiss et al. 1996], IQ [Andreasen et al. 1993;Posthuma et al. 2002;Reiss et al. 1996;Thompson et al. 2001;Willerman et al. 1991], handedness [Kertesz et al. 1990;Witelson and Goldsmith 1991], length [Piven et al. 1996], weight, socioeconomic status [Andreasen et al. 1990;Pearlson et al. 1989] and use of (neuroleptic) medication [Chakos et al. 1994;Chakos et al. 1998;Gur et al. 1998;Scheepers et al. 2001a;Scheepers et al. 2001b] all influence the size of focal brain structures. In none of the earlier studies patients and control groups were matched on all these factors, and medication status was often not taken into consideration. It is known from studies on subjects with schizophrenia that neuroleptics, which are also regularly used by autistic subjects, affect brain(part) volumes [Chakos et al. 1994;Gur et al. 1998]. Therefore, the use of medication-naive patients is important if one wants the relation between the autistic syndrome and brain volumes.

We compared brain scans of a group of 21 medication-naive high-functioning adolescents and young adults with PDD with those of 21 healthy volunteers, matched on gender, age, total IQ, verbal IQ, performance IQ, length, weight, handedness and parental education. Our aim was to determine (1) whether brain abnormalities are still present in adolescents and young adults with PDD, and if so, (2) whether these brain enlargements in patients are confined to the gray and/or the white matter and/or the cerebrospinal fluid, and (3) whether these enlargements are global or more prominent in some cortical volumes.

Subjects and methods

Subjects

Twenty-one medication-naive, high-functioning patients fulfilling the DSM-IV criteria of autism [American Psychiatric Association 1994] were recruited at the Leo Kanner Huis (an in- and out-patient clinic for patients with pervasive developmental disorders) and through advertising. Six patients did not have any cognitive or language delays at age three. To confirm clinical diagnosis, parental informants for all patients were interviewed with the ADI-R [Lord et al. 1994]. All patients fulfilled the ADI–R algorithm for both the social behavior and the communication subscores. Six patients scored just below the threshold for the ritualistic-repetitive behavior subscore (see table 1). Twenty-one healthy comparison subjects were recruited at a regional education center in Utrecht and were selected from the Utrecht Schizophrenia Project. Written informed consent was obtained for all subjects after the procedures had been fully explained. All subjects were between the ages of 15 and 25. Subjects with first degree relatives with a psychiatric disorder, a major medical or neurological illness, including epilepsy, alcohol or other drug dependence, head trauma in the past, or IQ below 80 were excluded. The presence or absence of psychopathological abnormalities was established in all subjects using questionnaires and a short version of the Comprehensive Assessment of Symptoms and History [Andreasen et al. 1992]. All subjects participated in a 45-minute MRI scanning session and a neuropsychological assessment in order to estimate full-scale WAIS-R IQ [Wechsler 1974].

Patients and healthy comparisons were matched for gender, age, IQ, height, weight, handedness, and for the socioeconomic status of their parents expressed as the highest completed level of education by either parents (see table 1 for demographics).

Brain MRI scans from all subjects were evaluated by two independent clinical neuroradiologists. No gross abnormalities were reported in any of the subjects.

MRI acquisition and prodecures

Magnetic resonance images were acquired on a Philips NT scanner operating at 1.5 T (Philips Medical Systems, Best, the Netherlands) in all subjects. A T1-weighted Three Dimensional - Fast Field Echo (3D-FFE: TE=4.6 ms, TR=30 ms, flip angle=30°, FOV=256x256 mm^2, in plane voxel size 1mm x 1mm) with 160-180 contiguous coronal 1.2 mm slices and a T2-weighted Dual Echo – Turbo Spin Echo (DE-TSE: TE1=14 ms, TE2=80 ms, TR=6350 ms, flip angle = 90°, FOV=256x256 mm^2, in plane voxel size 1mm x 1mm) with 120 contiguous coronal 1.6 mm slices of the whole head were used for the quantitative measurements -the T2-weighted sequence for measurement of the intracranial volume, the T1-weighted sequence for all other quantitative measurements. In

Table 1: Demographic data

	Patients (n=21)	Healthy comparisons (n=21)
Male/female participants, no	19/2	20/1
Age, mean ± SD (range), y	20.08 ± 3.10 (15.5 – 24.7)	20.28 ± 2.22 (17.3 – 24.8)
Total IQ, mean ± SD	114.90 ±19.18	112.62 ±10.20
Verbal IQ, mean ± SD	112.90 ±19.64	107.62 ±9.89
Performance IQ, mean ± SD	114.00 ±16.22	116.95 ±11.53
Height, mean ± SD, cm	180.62 ± 10.40	179.95 ± 7.37
Weight, mean ± SD, kg	70.14 ± 12.94	74.24 ± 9.10
Handedness (right/left), no	19/2	17/4
Parental education, mean ± SD, y	14.76 ± 2.00	13.52 ± 2.71
ADI: social deficits	19.62 ± 5.88	
ADI: abnormalities in communication	15.90 ± 3.74	
ADI: ritualistic-repetitive behavior	4.10 ± 2.66	

addition, a T2-weighted Dual Echo –Turbo Spin Echo (TE1=9 ms, TE2=100 ms, TR=2200 ms, flip angle=90°, FOV=250x250 mm^2) with 17 axial 5 mm slices and 1.2 mm gap of the whole head was acquired for clinical neurodiagnostic evaluation. Processing was done on the neuroimaging computer network of the Department of Psychiatry, which includes Hewlett Packard (Palo Alto, CA) Unix 9000 workstations, a compute server and Pentium III-equipped personal computers. Subject motion was quantified using an algorithm that compares the amount of artifact due to motion in the frequency encoding direction beside and above the head ('motornoise') [Schnack et al. 2002]. In addition, all scans were qualitatively rated on a four-point scale (0-3 points, with 0 being given to an unusable scan and 3 representing a MRI scan of excellent quality). All images were coded to ensure blindness for subject identification and diagnosis; scans were put into Talairach frame (no scaling) [Talairach and Tournoux 1988], and corrected for inhomogeneities in the magnetic field [Sled et al. 1998]. Quantitative assessments of the intracranial, total brain, cerebral gray and white matter (total brain excluding cerebellum and stem), lateral and third ventricles, and peripheral cerebrospinal fluid (CSF) volumes were performed based on histogram analyses and series of mathematical morphological operators to connect all voxels of interest, validated previously [Schnack et al. 2001a]. A plane through the fourth ventricle and the aqueduct limited the cerebellum. In lateral ventricle segmentation automatic decision rules bridged connections not detectable and prevented 'leaking' into cisterns [Schnack et al. 2001b].

Coronal slices clearly showing the anterior and posterior commissures limited the third ventricle; the upper boundary was a plane through the plexus choroideus ventriculi tertii perpendicular to the midsagittal slice. All images were checked after the measurements and corrected manually if necessary using Analyze TM [Robb 1995]. The interrater reliability of the volume measurements determined by the intraclass correlation coefficient in 10 brains was 0.95 and higher [Hulshoff Pol et al. 2002].
Frontal, parietal, temporal, and occipital lobes were manually demarcated on a brain image that served as a model. The modelbrain was selected earlier among 200 brain images of healthy subjects between 16-70 years of age [Mandl et al. 1999]. The frontal lobes were limited by the frontal pole, the lateral fissure, and the interhemispheric, the circular insular, the central, the olfactory, and the cingulate sulci. The parietal lobes were limited by the central, the interhemispheric, the circular insular, the subparietal, and the cingulate sulci, the lateral ventricles, and the lateral, and the parieto-occipital fissures. The temporal lobes were limited by the lateral, the circular insular, the anterior calcarine, and the interhemispheric sulci, the amygdala-hippocampal complex, the lateral ventricles, and the temporo-occipital notch. The occipital lobes were limited by the interhemispheric sulci, the parieto-occipital fissure, and the temporo-occipital notch (see figure 1). Brain images were registered to the model brain through the ANIMAL algorithm [Collins et al. 1996] to remove global differences in size and shape of the individual brains. The inverse of the transformation process registered the manual segmentations of the model brain to all subjects' brain images. The warped segments were visually checked. The gray and white matter segments from the individual brain images were used to divide model-based segmentations into gray and white matter.

Statistical analyses

All clinical data and brain volume measures were found to be normally distributed. In the lateral ventricle volumes there was one outlier in the patient group and one outlier in the comparison group. Therefore, these data were analyzed both with and without these subjects to determine if they contributed disproportionately to the results.
Differences in amount of artifact due to motion and qualitative scan ratings between patient and controls were investigated using a two-tailed t-test.
To examine whether brain volumes differed between the patients and the healthy comparison subjects, multiple analyses of variance (ANOVAs) were done with intracranial, total brain, frontal lobe, parietal lobe, temporal lobe, occipital lobe, gray and white matter of the cerebrum (total brain, excluding cerebellum and brainstem), gray and white matter of the four cortical lobes, cerebellum, and lateral and third ventricular volume as dependent variables and group (patients, healthy comparison subjects) as independent variable.

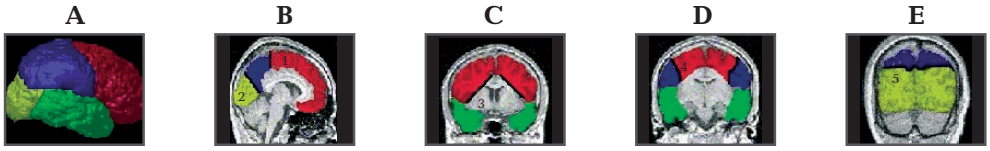


Figure 1

Lobar boundaries. Volumes of the individual cortical regions were manually traced on a standard brain in coronal direction using natural and, if necessary, artificial boundaries. Frontal lobe = red; parietal lobe = blue; temporal lobe = green, and occipital lobe = yellow. A: 3-D image of the brain; B: parasagittal view of the brain; C-E: coronal views; 1: As long as the central sulcus is not visible, a line is drawn, perpendicular to the AC-PC line from the deepest point of the marginal segment of the cingulate gyrus; 2: parieto-occipital fissure; 3: line between most lateral point of the cingulate sulcus and most medial point of the circular insular sulcus; 4: central sulcus, followed by a line between the deepest point of the central sulcus and the most medial point of the circular insular sulcus; 5: medially, the boundary is formed by the parieto-occipital fissure, which is followed to its most lateral point and from thereon a straight line, parallel to the AC-PC, is drawn to the lateral side of the brain.

Using ANCOVAs, analyses were repeated with intracranial volume as a covariate to examine whether volumetric differences in brain volume could be explained by differences in intracranial volume between patients and comparison subjects. In addition, total brain volume was used as a covariate to examine whether brain volumes were (dis)proportionately increased (or decreased).
In case of a significant finding between the two groups, MAN(C)OVAs were performed with the enlarged brain volumes as dependent variables, group as between-subject variable, and side (left, right) as within-subject variable, to examine whether differences in brain volumes were asymmetrical.
SPSS 9.0 statistical package for Windows [SPSS Inc, Chicago, III] was used for these analyses, with a 2-tailed alpha level of 0.05.

Results

No significant differences, either in the amount of artifact due to motion (t=1.62, p=0.12) or in qualitative rating of the MRI scans (t=0.35, p=0.73), were found between patients and controls.

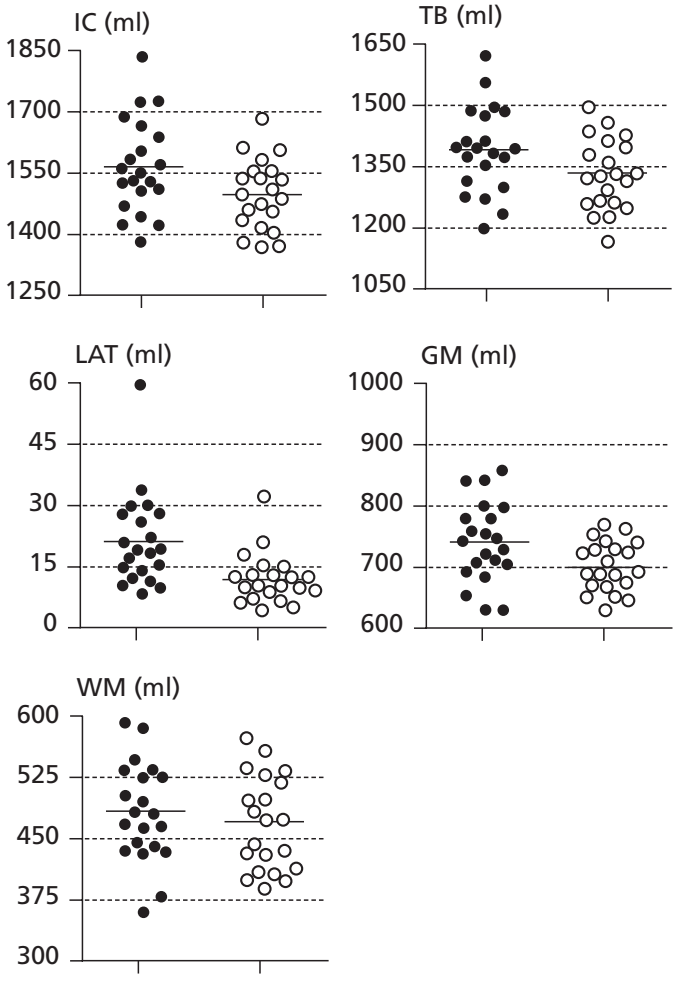
For mean (sd) values of the brain volumes see table 2. Intracranial, total brain, cerebral gray matter, frontal lobe, frontal gray matter, cerebellum and lateral and third ventricular volumes were significantly larger in patients compared to comparison subjects. Parietal, temporal and occipital gray matter volumes were all larger in patients, but these did not reach significance (see table 2 and figure 2). After correction for brain volume, lateral (F=9.29, df=1,39, p=0.004) and third (F=12.20, df=1,39, p=0.001) ventricular volumes remained significantly different between the two groups. Exclusion of the outliers in lateral ventricular volume from the data did not alter the findings (F=11.75, df=1,37, p=0.002).

Table 2: Absolute brain volumes (ml) of autistic and healthy comparison subjects

	Patients (n=21) Mean ± SD	Healthy comparisons (n=21) Mean ± SD	F df=1,40	p
Intracranium	1564.11 ±117.09	1494.74 ±87.77	4.72	0.04*
Total brain	1393.92 ±105.87	1333.33 ±86.61	4.12	0.05*
Cerebral gray matter	740.80 ±65.94	701.48 ±41.72	5.33	0.03*
Cerebral white matter	482.16 ±60.87	467.93 ±57.57	0.61	0.44
Frontal lobe	425.14 ±32.92	405.03 ±29.18	4.39	0.04*
Frontal gray matter	247.17 ±21.30	235.10 ±14.19	4.67	0.04*
Frontal white matter	177.96 ±19.14	169.93 ±18.97	1.87	0.18
Parietal lobe	217.77 ±21.60	208.57 ±15.77	2.49	0.12
Parietal gray matter	133.39 ±14.01	126.77 ±8.07	3.52	0.07
Parietal white matter	84.38 ±11.85	81.80 ±10.11	0.58	0.45
Temporal lobe	224.14 ±17.68	215.35 ±14.83	3.05	0.09
Temporal gray matter	157.17 ±12.68	150.43 ±9.48	3.80	0.06
Temporal white matter	66.97 ±11.10	64.92 ±9.05	0.43	0.52
Occipital lobe	116.17 ±13.43	109.33 ±10.94	3.27	0.08
Occipital gray matter	66.51 ±8.29	62.03 ±6.65	3.72	0.06
Occipital white matter	49.66 ±8.17	47.30 ±6.14	1.12	0.30
Cerebellum	155.67 ±10.94	148.72 ±10.01	4.62	0.04*
Lateral ventricles	21.08 ±11.57	11.87 ±6.11	10.40	0.003**
Third ventricle	0.96 ±0.41	0.57 ±0.22	15.76	<0.0001**

Figure 2

Scatterplots and mean values of volume of the intracranium (IC), total brain (TB), lateral ventricle (LAT), gray matter of the cerebrum (GM), and white matter of the cerebrum (WM) in 21 high-functioning medication-naive patients (black dots, left side of each graph) and 21 comparison subjects (open dots, right side of each graph).



There was no significant difference in any of the white matter volumes between patients and healthy comparisons. No significant interactions between side and group were found.

Discussion

This cross-sectional study compared brain morphology in 21 high-functioning patients with PDD between 15 and 25 years of age and 21 closely matched, healthy comparison subjects. Its main finding is a significant increase of approximately 5% in intracranial, total brain, cerebral gray matter, frontal gray matter, and cerebellum volume, and an increase of more than 70% in lateral and third ventricular volume in patients compared to comparison subjects. After correction for brain volume, lateral and third ventricular volume remained significantly enlarged in the patient group compared to the control group. Cerebral white matter volume did not differ significantly between the two groups. None of the enlarged brain areas showed a significant interaction between side and group.

The increase in intracranial, total brain, and cerebellum volume found in the present study is consistent with some earlier reports [Hardan et al. 2001a;Piven et al. 1995;Piven et al. 1996], but see [Aylward et al. 2002;Aylward et al. 1999;Carper et al. 2002;Courchesne et al. 2001;Haznedar et al. 2000;McAlonan et al. 2002;Rojas et al. 2002;Townsend et al. 2001]. These different results may be related to differences in characteristics between the patient group of the present study and the patient groups included in other studies. Although it is suggested that abnormality in brain development is a characteristic feature of autism regardless of IQ [Aylward et al. 2002], several studies revealed a significant positive correlation between brain volumes and intelligence measures (for example see [Andreasen et al. 1993;Posthuma et al. 2002;Reiss et al. 1996; Thompson et al. 2001;Willerman et al. 1991]). Thus, as was put forward by Akshoomoff et al. [Akshoomoff et al. 2002], increased brain volume may be found only in high-functioning autistic subjects. Indeed, studies reporting increased brain volume in autistic adolescents and adults have typically included high-functioning autistic subjects [Hardan et al. 2001a;Piven et al. 1995;Piven et al. 1996]. Furthermore, relatively small sample sizes [Aylward et al. 1999;Rojas et al. 2002;Townsend et al. 2001] may have caused type II error, due to lack of statistical power.

Interestingly, the increase in both lateral and third ventricular volume was disproportionately large and still present after correction for total brain volume. This finding has not been reported before in autistic subjects probably as other studies have typically not corrected brain volume [Courchesne et al. 1987;Gaffney et al. 1989;Howard et al. 2000;Piven et al. 1995;Townsend et al. 2001]. However, percent brain enlargement was 1.3-6.7% in those studies [Howard et al. 2000;Piven et al. 1995;Townsend et al. 2001], whereas ventricular enlargement was 28.9-153%. Thus, it seems that the results from those studies may indicate a disproportional ventricular enlargement as well.

The present study found an increase of 5% in gray - but not white- matter

volume in high-functioning patients with PDD. This increase was proportional to the increase in brain volume and was evenly distributed among the four cortical lobes. However, the increase in frontal gray matter volume was the only one to reach significance, whereas gray matter volumes of the other three lobes (i.e. parietal, temporal, and occipital) only reached trend level. This is most likely due to the frontal lobe being the largest lobe. Furthermore, it should be stressed that the method we used provides an approximation of the separate lobar volumes, as the boundaries were not directly measured in each individual brain, making definite statements about lobar volume changes presumptuous. Previous studies reported different results on gray and white matter and lobar volume differences between autistic patients and controls, which is most likely due to (1) a different method, used for determining lobar volumes [Piven et al. 1996], which has been found to reveal different volumetric results [Tisserand et al. 2002], (2) different composition of the patient and the control groups – i.e. inclusion of only patients with Asperger syndrome [McAlonan et al. 2002] or inclusion of patients with mental retardation [Carper et al. 2002;Courchesne et al. 2001], and (3) relatively small sample sizes [Townsend et al. 2001]. Thus, the significantly increased cerebral gray matter volume found in the present study could have been due to the inclusion of high-functioning patients only and IQ-matched controls. Indeed, it is known from the literature that gray matter volume and density are positively correlated with IQ [Andreasen et al. 1993;Posthuma et al. 2002;Thompson et al. 2001]. Furthermore, our findings of increased total brain and (frontal) gray matter volume are consistent with post-mortem studies, finding increased brain weight and size in 19-50% of adults with autism [Bailey et al. 1998;Courchesne et al. 1999], but see [Kemper and Bauman 1998]. Moreover, the increases in the gray matter volume – particularly in the frontal lobe - are consistent with the reported abnormally thickened frontal cortices in three out of six autistic brains [Bailey et al. 1998]. Furthermore, a post-mortem study reported more, but smaller minicolumns in patients with autism [Casanova et al. 2002], pointing towards an increase in cortical surface area. The absence of white matter findings in our study is also in agreement with post-mortem research, finding no myelination abnormalities [Bauman 1991;Bauman and Kemper 1994], although in one autistic adult increased number of white matter neurons was found [Bailey et al. 1998]. As to the nature and timing of the underlying pathophysiology of the brain enlargement in autism one can only speculate. Our results suggest that the increased brain volume in autism is due to enlargement of the gray –but not white- matter volume. Decreased elimination of neural processes -including programmed cell death (apoptosis), axonal pruning, and dendritic degeneration-, as well as increased neurogenesis have been suggested to occur in autism [Piven et al. 1995;Piven et al. 1996]. It is known from the literature that serotonin can block the processes of apoptosis (for review, see [Azmitia 2001]). Thus, it might be

possible that the reported hyperserotonemia in autism [Leboyer et al. 1999;Veenstra-VanderWeele et al. 2002] blocks the process of apoptosis excessively. Furthermore, in Rett's syndrome, another pervasive developmental disorder, loss of serotonin produces a reduction in the thickness of gray matter, not a reduction of myelin [Azmitia 2001]. Thus, it might be possible that hyperserotonemia in autism produces increased thickness of the gray matter without affecting the white matter. Regarding the enlarged ventricles, one possible explanation might be that subcortical structures in the neighborhood of the ventricles are disproportionately smaller in autism. However, to date, two studies investigated the basal ganglia, one reporting an increase in caudate volume [Sears et al. 1999] and the other in putamen-globus pallidus volume [Herbert et al. 2003]. On the other hand, a recent study investigated the thalamus and reported decreased thalamic volume in those autistic subjects who had large brains [Tsatsanis et al. 2003], but see [Herbert et al. 2003]. Another possible explanation would be that brain volumes in patients at younger ages are more than 5 % enlarged and thus, brain tissue decreases relatively more rapidly in patients compared to controls, resulting in disproportionately increased ventricular volume. Further research, with a longitudinal set-up, can address these options. Apart from the unresolved nature of the underlying process, the timing is unclear as well. It is unknown whether the abnormal development starts in the prenatal period or whether it is a postnatal process. Clinical data reveal that head size at birth seems to be normal in most cases of autism [Courchesne et al. 2001;Lainhart et al. 1997;Stevenson et al. 1997], suggesting that there is an increased rate of postnatal brain growth in autism compared to comparison subjects [Hardan et al. 2001a]. Other evidence of postnatal aberrant brain growth is suggested by abnormally elevated brain neurotrophins and neuropeptides (brain-derived neurotrophic factor, neurotrophin-4, vasoactive intestinal peptide, calcitonin-related gene peptide) found in neonatal blood spots of individuals who later developed autism and mental retardation [Nelson et al. 2001]. Abnormal elevations may result in unusual growth abnormalities [Courchesne et al. 2001]. However, recently reported abnormalities in cytoarchitectural arrangement (i.e. the minicolumns) may originate during the neurogenesis of the cell columns and take place in the prenatal period [Casanova et al. 2002]. Other post-mortem research postulated a prenatal timing of the abnormalities as well [Bailey et al. 1998;Bauman 1991;Kemper and Bauman 1998;Kemper and Bauman 1993]. Thus, the available data are inconclusive as to the exact nature and onset of abnormal development. Future (post-mortem) research is necessary to clarify the underlying mechanisms of increased gray matter volume and disproportional enlarged ventricles in patients found in the present study.

This study was limited in several aspects. These limitations should be taken into consideration when interpreting its findings. First and foremost, its relatively

small sample size. The present findings of increased gray matter volume in high-functioning patients with PDD need to be replicated in a larger sample size. Second, because this study was restricted to high-functioning patients, results may not be representative of those in mentally retarded patients, representing about 75% of all autistic individuals. Third, the present study only measured volumes of (large parts of) the brain. It remains to be investigated whether focal, subcortical gray matter structures are abnormal in these patients.

Conclusions

This study found proportional brain enlargements of 5% in high-functioning patients with PDD, including intracranial, total brain, cerebellum and cerebral gray matter volume –with no clear and robust pattern of specific lobar volumetric changes-, and disproportional enlargements of lateral and third ventricular volumes. Thus, at least in high-functioning patients with PDD, brain enlargement may still be present in adult life.

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Chapter 5

No evidence for preferential involvement of medial temporal lobe structures in high-functioning autism

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Abstract

Background: Autism is a neurodevelopmental disorder associated with a slight increase in overall brain volume. There has been some suggestion that medial temporal lobe structures may be preferentially involved in this disorder, although results have not always been consistent. Here, we investigate amygdala and hippocampus volumes in medication-naive subjects with high-functioning autism. **Methods:** Whole-brain MRI scans were acquired from 42 subjects with autism and 42 closely matched, healthy control subjects. **Results:** Amygdala volume did not differ significantly between subjects with autism and controls. An increase in hippocampus volume was proportional to an increase in overall brain volume. **Conclusions:** These results argue against preferential involvement of medial temporal lobe structures in autism, at least in high-functioning medication-naive individuals.

Introduction

Autism is a neurodevelopmental disorder, defined by the presence of marked social deficits, specific language abnormalities, and stereotyped, repetitive behaviors. Medial temporal lobe structures have been implicated in this disorder, due to their role in social behavior, facial recognition, and memory processing, all of which have been implicated in autism in behavioral and functional imaging studies [Cody et al. 2002]. Neuropathological studies have reported abnormally small and densely packed cells in the amygdala and the hippocampus of autistic subjects [Kemper and Bauman 1993]. However, volumetric MRI studies have been less consistent as they have reported increases, decreases, and unchanged amygdala and hippocampal volumes in autistic subjects compared to controls [Cody et al. 2002]. However, recently the involvement of medial temporal lobe structures in autism has been questioned, due to the absence of impaired social behavior in patients with bilateral amygdala damage [Amaral et al. 2003].
In this paper, we address this issue by investigating amygdala and the hippocampus volumes in a large sample of medication-naive, high-functioning subjects with autism and Asperger's syndrome. Previously, we reported an increase in overall brain volume in these subjects [Palmen et al. 2004;Palmen et al. in press]. Here, we investigate whether medial temporal lobe structures are preferentially affected.

Methods

Subjects

Forty-two medication-naive, high-functioning children and adolescents meeting DSM-IV criteria for autism (n= 21) or Asperger's syndrome (n= 21) were included in the present study (mean age 15.6 ± 5.3 years, mean full IQ 110.7 ± 16.9, 2 females, and 3 left-handed subjects). Clinical diagnosis was confirmed by parent interview for all participants using the ADI-R [Lord et al. 1994]. The control group consisted of 42 typically developing children and adolescents matched for age, gender, IQ, height, weight, handedness, and parental education (mean age 15.3 ± 5.4 years, mean full IQ 107.6 ± 13.4, 1 female subject, and 6 left-handed subjects) [Palmen et al. 2004;Palmen et al. in press]. Written informed consent was obtained for all subjects after the procedures had been fully explained. Subjects with first-degree relatives with a psychiatric disorder, a major medical or neurological illness, or full IQ below 80 were excluded.

MRI acquisition and procedures

Magnetic resonance images were acquired on a Philips NT scanner operating at 1.5 T (Philips Medical Systems, Best, the Netherlands). Two near-identical scanning protocols were used, that differed only in slice thickness. Both included a T1-weighted whole-brain 3D fast field echo scan, with either 1.2 or 1.5 mm contiguous coronal slices, as well as a T2-weighted dual echo turbo spin echo scan with 1.5 or 3.0 mm contiguous coronal slices. Twenty-one subjects with autism were scanned with each protocol, as well as 21 controls. Ten control subjects were scanned with both protocols, in order to compare volumetric measures obtained from the two protocols. Intraclass correlation coefficients (ICCs) were greater than 0.90 for all measures. Semi-automated, histogram based algorithms were used to estimate intracranial, total brain, ventricle, cerebellar and gray and white matter volume, as previously reported [Palmen et al. 2004;Palmen et al. in press]. Medial temporal lobe structures were traced manually by a single experienced rater (HN), blind to subject identity. Scans were randomly flipped over the Y-axis to ensure rater blindness to laterality. Amygdala and hippocampus were outlined in contiguous coronal slices in an anterior - posterior direction, according to previously published criteria [Baaré et al. 2001]. The only adjustment to the segmentation criteria involved the first slice of the amygdala. Here, the amygdala was first traced in the first slice where it was discernible after the anterior commissure no longer appeared as a continuous tract. Ten scans were duplicated and randomly intermixed with the dataset, in order to estimate intrarater reliability. ICCs were 0.83 for left amygdala, 0.84 for right amygdala, 0.86 for left hippocampus and 0.91 for right hippocampus.

Statistical analyses

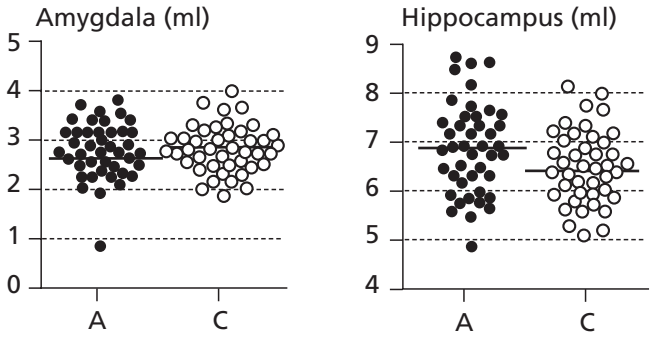
SPSS 9.0 statistical package for Windows (SPSS Inc, Chicago, III) was used for all statistical analyses. All clinical data and brain volume measures were normally distributed.

Independent sample t-tests were performed with amygdala and hippocampal volumes as dependent variables. To control for overall brain volume, analyses were repeated with intracranial and total brain volume separately as covariates. In the patient group, there was one outlier in the volume of the amygdala. Therefore, data were analyzed both with and without this subject.

Results

Scatterplots of amygdala and hippocampus volume are depicted in figure 1.
No significant difference in amygdala volume was found (t= -0.01, p= 0.99). Exclusion of the outlier did not alter the results significantly (t= 0.43, p= 0.67). Absolute hippocampus volumes were enlarged (t= 2.21, p= 0.03 for total hippocampal volume; t= 2.04, p= 0.04 for left hippocampus; t= 2.08, p= 0.04 for right hippocampus), although these findings were no longer significant after co-varying for intracranial or total brain volume.

Figure 1:
Scatterplots of volumes of the amygdala (left figure) and the hippocampus (right figure) in 42 autistic subjects (A) and 42 healthy controls (C).



Discussion

In this cross-sectional study, we compare the volumes of medio-temporal lobe structures in 42 medication-naive, high-functioning subjects with autism and 42 matched control subjects. Volume of the amygdala did not differ significantly between autistic subjects and controls. Although there was an absolute increase in hippocampus volume, this increase was proportional to an increase in overall brain volume.
Previous reports of medial temporal lobe volumes in autism have not been entirely consistent, as both increases and decreases in volume have been reported [Cody et al. 2002]. This study included a relatively large sample of subjects with autism, all of whom were high-functioning and medication-naive.

This may partially explain differences with previous studies that have typically included smaller samples, as well as low-functioning and medicated individuals. Furthermore, not all studies have included estimates of overall brain volume to investigate whether volume changes in temporal lobe structures were proportional to changes in brain volume (see [Cody et al. 2002]). Furthermore, differing segmentation procedures may have contributed to the variation in findings [Brierley et al. 2002;Geuze et al. 2004].
In short, these findings do not support the preferential involvement of medial temporal lobe structures in high-functioning autism. However, structural MRI measures we have investigated here may not reflect changes in brain function. As such, we cannot exclude possible functional impairments in the medial temporal lobe.

Conclusion

We find no support for a preferential involvement of medial temporal lobe structures in autism, in a sample of medication-naive, high-functioning subjects. However, these volumetric, anatomically based measures do not prohibit a functional involvement of these structures in this disorder.

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Chapter 6

Brain anatomy in non-affected parents of autistic probands: an MRI study

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Abstract

Background: Autism is a neurodevelopmental disorder with an estimated genetic origin of 90%. Previous studies have reported an increase in brain volume of approximately 5% in autistic subjects, especially in children. If this increase in brain volume is genetically determined, biological parents of autistic probands might be expected to show brain enlargement, or at least intracranial enlargement, as well. Identifying structural brain abnormalities under genetic control is of particular importance as these could represent endophenotypes of autism. **Methods:** Using quantitative anatomic brain magnetic resonance images, volumes of intracranial, total brain, frontal, parietal, temporal, and occipital lobe, cerebral and cortical gray and white matter, cerebellum, lateral ventricle, and third ventricle were measured in biological, non-affected parents of autistic probands (19 couples) and in healthy, closely matched control subjects (20 couples). **Results:** No significant differences were found between the parents of the autistic probands and healthy control couples in any of the brain volumes. Adding gender as a factor in a second analysis did not reveal a significant interaction effect of gender by group. **Conclusions:** Biological, non-affected parents of autistic probands do not show brain enlargements. As the intracranium is not enlarged, it is unlikely that the brain volumes of the parents of autistic probands have originally been enlarged and have been normalized. Thus, increased brain volume in autism might be caused by the interaction of paternal and maternal genes, possibly with an additional effect of environmental factors, or increased brain volumes might reflect phenotypes of autism.

Introduction

Autism is an etiologically complex, neurodevelopmental disorder, defined by the presence of marked social deficits, specific language abnormalities, and stereotyped, repetitive behaviors [American Psychiatric Association 1994]. It has been estimated that over 90% of the etiology is derived from genetic factors [Bailey et al. 1995], likely of oligogenic etiology [Risch et al. 1999]. Parents share specific behavioral and cognitive characteristics with their autistic probands, suggesting that these characteristics are likely genetic in nature. Significant social abnormalities, such as schizoid personality traits [Wolff et al. 1988], lack of friendships and aloofness [Lainhart et al. 2002;Piven et al. 1997a] seem to affect a substantial minority (25-30%) of parents. Likewise, most studies reported more communication impairments in parents of autistic probands compared to controls [Gillberg et al. 1992;Lainhart et al. 2002;Landa et al. 1992;Piven et al. 1997a]. Stereotyped, rigid behavior, the third key feature of autism, seems to occur either in only a small minority of parents [Lainhart et al. 2002;Piven et al. 1997b], or not at all [Wolff et al. 1988]. Finally, cognitive impairments, such as deficits in theory of mind [Baron-Cohen and Hammer 1997], weak central coherence [Happé et al. 2001], and executive function deficits [Hughes et al. 1997], seem to run in families with autistic probands. In addition, parents of autistic probands show increased rates of other genetically determined psychiatric disorders, such as depression [Piven and Palmer 1999], anxiety disorder [Piven and Palmer 1999], and obsessive compulsive disorder [Bolton et al. 1998]. Neurobiological studies revealed such a familial pattern as well. Increased whole blood serotonin [Leboyer et al. 1999], decreased levels of Reelin [Fatemi et al. 2002], and dysregulated amino acid metabolism [Aldred et al. 2003] were found in both autistic probands and their parents. In addition, previous studies have shown that head circumference, known to be genetically determined [Weaver and Christian 1980], is above the 97th percentile (i.e. macrocephaly) in approximately 20% of autistic individuals [Stevenson et al. 1997], and in 20 – 62% of parents of autistic subjects, especially parents of autistic probands with increased head circumference [Fidler et al. 2000; Stevenson et al. 1997]. Likewise, brain volume, with a genetically explained variance of 90% [Baaré et al. 2001a], has also been found to be enlarged in autistic individuals, especially in children [Aylward et al. 2002;Courchesne et al. 2001;Palmen et al. in press a;Sparks et al. 2002]. The present study is, to our knowledge, the first to investigate brain volumes in biological parents of autistic probands. Identifying structural brain abnormalities under genetic control is of particular importance because these could represent endophenotypes of autism. Endophenotypes are defined as traits that carry genetic loading and which are related indirectly to the classic behavioral symptoms as defined in DSM-IV or ICD-10 [Skuse 2001]. In complex heterogeneous disorders such as autism,

endophenotypes are presumed to be more proximal to gene action and therefore could aid in the identification of susceptibility genes. We compared brain scans of 19 biological parent couples of an autistic proband with those of 20 healthy married control couples, matched on age, gender, IQ, height, weight, handedness and education. Inclusion of both parents ensures the inclusion of the obligate carrier(s) and by comparing parents of autistic subjects with healthy control couples one can correct for assortative mating –the tendency for mated pairs to be more similar for some traits than would be expected of the choice of a partner that occurred at random. The aim was to determine (1) whether brain enlargement is present in parents of autistic probands with known increased brain volumes [Palmen et al. in press a;Palmen et al. in press b], and if so, (2) whether this enlargement is found to the same extent in fathers and in mothers, and (3) whether enlargement is present in the same regions as in the autistic probands themselves.
It was realized that, although some studies, including our own, reported increased brain volume in autistic adolescents and adults [Hardan et al. 2001;Palmen et al. in press b;Piven et al. 1995] other studies did not [Aylward et al. 1999;Courchesne et al. 2001;Haznedar et al. 2000;Rojas et al. 2002], suggesting that the increase in brain volume might only be a passing thing. In that case, brain enlargement that would be genetic in origin, would not be seen in parents of autistic probands as they would have outgrown their brain enlargement. However, as it is in the early stages of brain development that the intracranial volume expands under the influence of brain growth [O'Rahilly and Muller 1992;Sgouros et al. 1999], intracranial volume would be expected to be enlarged in the parents of autistic probands if brain enlargement in autism is an endophenotype.

Methods

Participants

Forty-two biological parent couples with an autistic proband (autism parent couples) were asked to participate in the present study. All were parents of autistic subjects that had been previously included in studies of structural brain abnormalities in autistic subjects [Palmen et al. in press a;Palmen et al. in press b]. For 30 autistic probands both parents were able and willing to participate in the present study. After matching on demographic variables with the control sample, 19 autism parent couples –17 of which had an autistic proband with a brain volume larger than the control mean- remained to participate in the present study. The presence or absence of psychopathological abnormalities was established in all autism parent couples using questionnaires and a short

version of the Comprehensive Assessment of Symptoms and History [Andreasen et al. 1992]. Subjects with a major medical or neurological illness, including epilepsy, alcohol or other drug dependence, head trauma in the past, or IQ below 80 were excluded. After written consent was obtained, all autism parent couples participated in a 45-minute MRI scanning session. IQ was estimated with the aid of a short version of the Groningen Intelligence Test (GIT) [Luteijn and van der Ploeg 1983]. As the GIT is comparable to the WAIS-R and not to the more recent WAIS-III, all subjects scored on average 10 points higher than would have been expected when tested with the WAIS-III. Twenty healthy, married control couples were drawn from the database of the Utrecht Schizophrenia Project. The control subjects had been recruited previously [Appels et al. 2003] and had already been extensively screened to exclude psychopathology in themselves as well in their first-degree relatives and to exclude major medical or neurological illness, including epilepsy and alcohol or other drug abuse. The control subjects had undergone the same scanning session and the GIT had been used for IQ determination. The procedure was approved at the institutional review board of the University Medical Center in Utrecht, Netherlands. The final sample, matched on age, gender, IQ, height, weight, handedness, and educational level consisted of 19 autism parent couples and 20 healthy control couples (see table 1 for demographics).

Table 1: Demographic data

	APC (n=38)	**HCC (n=40)**
Male/female participants, no	19/19	20/20
Age, mean ± SD (range), y	50.27 ± 3.79 (44.3-58.8)	52.01 ± 4.14 (41.8-59.9)
IQ, mean ± SD	117.50 ±10.52	117.85 ±12.56
Height, mean ± SD, cm	172.95 ± 9.41	174.93 ± 8.84
Weight, mean ± SD, kg	74.26 ± 13.59	75.33 ± 12.01
Handedness (right/left), no	34/4	38/2
Education, mean ± SD, y	13.71 ± 2.51	13.83 ± 2.24

APC: autism parent couples; HCC: healthy control couples

MRI acquisition and procedures

Magnetic resonance images were acquired on a Philips NT scanner operating at 1.5 T (Philips Medical Systems, Best, the Netherlands) in all subjects. A T1-weighted Three Dimensional - Fast Field Echo (3D-FFE: TE=4.6 ms, TR=30 ms, flip angle=30°, FOV=256x256 mm^2) with 160-180 contiguous coronal 1.2 mm slices and a T2-weighted Dual Echo – Turbo Spin Echo (DE-TSE: TE1=14 ms, TE2=80 ms, TR=6350 ms, flip angle = 90°, FOV=256x256 mm^2) with 120 contiguous coronal 1.6 mm slices of the whole head were used for the quantitative measurements -the T2-weighted sequence for measurement of the intracranial volume, the T1-weighted sequence for all other quantitative measurements. In addition, a T2-weighted Dual Echo –Turbo Spin Echo (TE1=9 ms, TE2=100 ms, TR=2200 ms, flip angle=90°, FOV=250x250 mm^2) with 17 axial 5 mm slices and 1.2 mm gap of the whole head was acquired for clinical neurodiagnostic evaluation. Processing was done on the neuroimaging computer network of the Department of Psychiatry. All images were coded to ensure blindness for subject identification and diagnosis; scans were put into Talairach frame (no scaling) [Talairach and Tournoux 1988], and corrected for inhomogeneities in the magnetic field [Sled et al. 1998]. Quantitative assessments of the intracranial, total brain, cerebral gray and white matter (total brain excluding cerebellum and stem), lateral and third ventricles, and peripheral cerebrospinal fluid (CSF) volumes were performed based on histogram analyses and series of mathematical morphology operators to connect all voxels of interest, developed and validated previously [Schnack et al. 2001a]. A plane through the fourth ventricle and the aquaduct limited the cerebellum. In lateral ventricle segmentation automatic decision rules bridged connections not detectable and prevented 'leaking' into cisterns [Schnack et al. 2001b]. Coronal slices clearly showing the anterior and posterior commissures limited the third ventricle; the upper boundary was a plane through the plexus choroideus ventriculi tertii perpendicular to the midsagittal slice. All images were checked after the measurements and corrected manually if necessary using DISPLAY [Pruessner et al. 2000]. The interrater reliability of the volume measurements determined by the intraclass correlation coefficient in 10 brains was 0.95 and higher [Hulshoff Pol et al. 2002].

Frontal, parietal, temporal, and occipital lobes were manually demarcated on a brain image that served as a model. The modelbrain was selected earlier among 200 brain images of healthy subjects between 16-70 years of age [Mandl et al. 1999]. The boundaries of the cortical lobes, extensively described in [Palmen et al. in press b], were set as follows. The frontal lobes were limited by the frontal pole, the lateral fissure, and the interhemispheric, the circular insular, the central, the olfactory, and the cingulate sulci. The parietal lobes were limited by the central, the interhemispheric, the circular insular, the subparietal, and the cingulate sulci, the lateral ventricles, and the lateral, and the parieto-occipital

fissures. The temporal lobes were limited by the lateral, the circular insular, the anterior calcarine, and the interhemispheric sulci, the amygdala-hippocampal complex, the lateral ventricles, and the temporo-occipital notch. The occipital lobes were limited by the interhemispheric sulci, the parieto-occipital fissure, and the temporo-occipital notch. Brain images were registered to the model brain through the ANIMAL algorithm [Collins et al. 1996] to remove global differences in size and shape of the individual brains. The inverse of the transformation process registered the manual segmentations of the model brain to all subjects′ brain images. The gray and white matter segments from the individual brain images were used to divide model-based segmentations into gray and white matter.

Statistical analyses

All clinical data and brain volume measures were found to be normally distributed. The only variable that was skewed to the right was the educational level. In the lateral ventricle volumes there was one outlier among the autism parent couples and one among the healthy control couples. In the cerebellum volumes, there was one outlier among the autism parent couples, whereas in the cerebral gray matter volume there was one outlier among the healthy control couples. Therefore, these data were analyzed both with and without these subjects to determine if they contributed disproportionately to the results.

To examine whether brain volumes differed between the autism parent couples and the healthy control couples, multiple analyses of variance (ANOVAs) were done with intracranial, total brain, frontal lobe, parietal lobe, temporal lobe, occipital lobe, gray and white matter of the cerebrum, gray and white matter of the four cortical lobes, cerebellum, and lateral and third ventricular volume as dependent variables and group (autism parent couples, healthy control couples) as independent variable. All analyses were repeated with intracranial volume as a covariate in multiple analyses of covariance (ANCOVAs) to examine whether volumetric differences in brain volumes could be explained by differences in intracranial volume between the autism parent couples and the healthy control couples.

A second analysis was performed with gender as an additional factor in order to look for possible interaction effects of gender by group.

SPSS 9.0 statistical package for Windows (SPSS Inc, Chicago, III) was used for these analyses, with a 2-tailed alpha level of 0.05.

Results

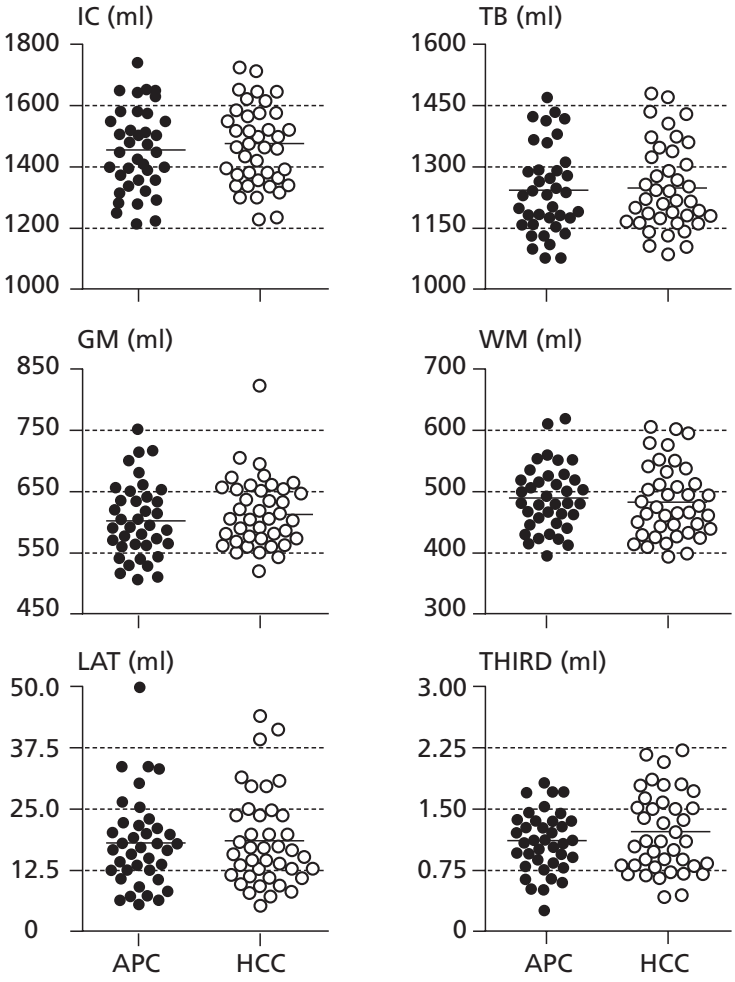
For mean (sd) values of the brain volumes see table 2 and figure 1. No significant differences in any of the brain volumes were found between the autism parent couples and the healthy control couples. Using intracranium as a covariate did not significantly alter the results. Exclusion of the outliers did not significantly alter the results.
Adding gender as a factor in a second analysis did not reveal any significant interactions of gender by group.

Table 2: Absolute brain volumes (ml)

	APC (n=38) Mean ± SD	HCC# (n=40) Mean ± SD	F# df=1,76	p
Intracranium	1454.81 ± 133.51	1473.84 ± 128.77	0.411	0.52
Total brain	1240.73 ± 107.39	1251.10 ± 104.99	0.186	0.67
Cerebral gray matter	600.09 ± 60.94	611.82 ± 57.13	0.760	0.39
Cerebral white matter	487.56 ± 53.82	482.49 ± 60.41	0.151	0.70
Frontal gray matter	195.37 ± 20.40	198.91 ± 17.59	0.669	0.42
Frontal white matter	174.19 ± 20.28	175 .21 ± 23.49	0.042	0.84
Parietal gray matter	108.48 ± 11.18	109.05 ± 8.26	0.065	0.80
Parietal white matter	84.24 ± 10.23	85.19 ± 12.80	0.128	0.72
Temporal gray matter	131.88 ± 11.33	132.19 ± 10.64	0.016	0.90
Temporal white matter	71.87 ± 8.32	70.29 ± 11.76	0.463	0.50
Occipital gray matter	53.78 ± 7.14	54.35 ± 4.90	0.169	0.68
Occipital white matter	50.45 ± 7.16	48.92 ± 6.68	0.135	0.25
Cerebellum	139.24 ± 12.90	142.77 ± 11.97	1.57	0.21
Lateral ventricles	17.82 ± 9.38	18.18 ± 9.68	0.027	0.87
Third ventricle	1.08 ± 0.38	1.19 ± 0.48	1.28	0.26

For one control subject scan quality was insufficient to reliably separate gray and white matter. Thus, in the analyses of gray and white matter, degrees of freedom were 1,75.

Figure 1:
Scatterplots of 19 parent couples with an autistic proband (n=38, black dots, left side of each graph) and 20 healthy control couples (n=40, open dots, right side of each graph) of intracranial (IC), total brain (TB), cerebral gray matter (GM), cerebral white matter (WM), lateral ventricle (LAT), and third ventricle (THIRD) volume. APC: autism parent couples, HCC: healthy control couples



Discussion

This cross-sectional study compared brain volumes in 19 non-affected parent couples of an autistic proband and 20 healthy control couples, matched on gender, age, IQ, height, weight, handedness and educational level. Its main finding is an absence of significant differences in any of the brain volumes - including the volume of intracranium; total brain; gray and white matter of the cerebrum; frontal, temporal, parietal, and occipital gray and white matter; cerebellum; third and lateral ventricle - between the autism parent couples and the healthy control couples. The differences in brain volumes remained not significant after correction for intracranial volume. Adding gender as a factor in the analysis did not reveal any significant interactions of gender by group.

Both autism and brain volume are highly genetically determined, and brain enlargement had been reported in the autistic probands of the present autism parent couples [Palmen et al. in press a;Palmen et al. in press b]. However, no brain enlargement was found in the autism parent couples themselves, when compared to the healthy control couples. Despite ample evidence for genetic factors in autism, the illness does not show simple genetic transmission. Its pathogenesis likely involves multiple genetic and environmental effects [Bailey et al. 1995], with possible inheritance of risk factors from both parents [Cook, Jr. Et al. 1997;Hallmayer et al. 1996]. In other words, autism is likely the outcome of multiple selections for both genetic and environmental factors that result in brain abnormalities. Parents of autistic probands would be expected to share some, but not all, of these factors. Thus, it might be possible that cerebral anatomical abnormalities represent a non-inherited factor which, combined with genetic risk for autism, increases the chance that an individual will manifest the disorder. Such mechanisms might explain why autism parent couples show brain volumes that are no different from those of controls. In addition, the standard deviations of the brain volumes of the autism parent couples were comparable to those of the healthy control couples, which is contrary to the consistently reported larger standard deviations in brain volumes of autistic subjects compared to controls [Hardan et al. 2001;Palmen et al. in press a; Palmen et al. in press b;Piven et al. 1995]. This finding might strengthen the idea of ‘‘normal’’ brain volumes of autism parent couples.

The present study is, to the best of our knowledge, the first to investigate brain volumes in parents of autistic probands. Therefore, no comparison with earlier findings can be made. However, in schizophrenia, another highly heritable (estimated at 80%) psychiatric disorder [Cannon et al. 1998], studies of brain volumes in first-degree relatives have been performed. In short, twin and singleton sibling studies suggest the involvement of genetic and disease-related (possibly nongenetic) factors in the decrease in brain volume, found in patients [Baaré et al. 2001b]. Decrease in white matter volume seems to reflect the

increased genetic risk to develop schizophrenia, whereas the decrease in gray matter volume is related to environmental risk factors [Hulshoff Pol et al. 2004]. Lateral ventricular enlargement is predominantly influenced by environmental factors [Ohara et al. 1998], although genetic factors may be involved too [Baaré et al. 2001b]. Studies including only parents of schizophrenic offspring are less clear. No reduction in brain volume [McDonald et al. 2002], and either increased ventricular [Sharma et al. 1998] or normal ventricular volumes [McDonald et al. 2002] were found. Thus, compared to parent studies, twin studies seem more suitable for detecting structural brain abnormalities under genetic control in psychiatric disease, although the shared environment of the affected and the unaffected co-twin/sibling might have accounted partly for the larger resemblance in brain abnormalities compared to parents and schizophrenic probands [Sullivan et al. 2003].

The present study did not find any brain enlargements in the autism parent couples. One hypothesis is that both paternal and maternal genes, and probably additional environmental factors as well, are necessary to cause the brain abnormalities as found in autism. On the other hand, it might be possible that volumetric brain abnormalities represent phenotypes of autism and thus will only be found in subjects diagnosed with autism. Twin and sibling studies may be better suitable to assess this hypothesis. Another theory is based on the assumption that brain abnormalities in autism become less pronounced with age and will eventually disappear [Courchesne et al. 2001]. Taken together with the reported decrease in symptom severity with age [Piven et al. 1996], it might be hypothesized that the autism parent couples, some of which may have been diagnosed with autism in childhood, have 'outgrown' the autistic symptoms and the brain abnormalities. This hypothesis would explain the reported increased head circumference in a subset of parents of autistic probands [Fidler et al. 2000;Stevenson et al. 1997]. However, although we only had head circumference data on the autism parent couples (mean: 57 cm, sd 2 cm) and not on the healthy control couples, the highly significant positive correlations between head circumference and both intracranium and total brain volume in the autism parent couples ($r = 0.740$, $p < 0.0001$, respectively $r = 0.734$, $p < 0.0001$) makes it very unlikely, that -considering the absolute absence of difference in brain volume between autism parent couples and healthy control couples- a significant increase in head circumference of autism parent couples would have been found. In addition, if brain enlargement would have been present in the autism parent couples at a younger age, one would have expected the increased intracranial volumes to remain as well, since these volumes are considered stable after the age of five [O'Rahilly and Muller 1992]. In addition, ventricular volumes would be expected to be enlarged as well, in compensation to the extra loss of brain tissue. As neither the intracranial nor the ventricular volumes were increased in the present autism parent couples sample, and none of the autism

parent couples had officially been diagnosed with autism in childhood, this last theory seems rather unlikely. Thus, increased brain volumes in autism might be caused by the interaction of parental and maternal genes, possibly with an additional effect of environmental factors, or represent phenotypes of autism.

Limitations

This study was limited in several aspects. These limitations should be taken into consideration when interpreting its findings. First, the sample size, although considerable in comparison to the statistical power needed to detect significant differences in patients with autism, may have been too small to detect differences between parents of autistic probands and healthy control couples, especially when considering that one or both parent(s) may contribute to the genotype in the offspring. However, as the means and standard deviations of the brain volumes of the two groups are similar, it is unlikely that the results will change significantly when larger samples are being included. Using the present brain volumes in a power analysis, 2600 subjects would be needed to detect a significant difference between the two groups. Second, all autistic probands were high-functioning. As has been suggested previously [Szatmari et al. 1998], the genetic mechanisms of higher and lower functioning autistic subjects may be different. Therefore, the generalizability of the present data may be limited with respect to lower functioning autistic patients. Third, only brain volumes were measured in the present autism parent sample. It would have been interesting to have measures on the broader phenotype and their possible relation to brain volumes, as the broader phenotype has been suggested to occur in some parents of autistic probands [Gousse et al. 2002]. Fourth, global brain volumes have been measured in this study. Therefore, no inferences can be made as to whether focal brain structures (e.g., the amygdala) may be related to the genetic risk to develop autism. Fifth, only parents were included in the present study. Sibling and twin studies may be more suitable for detecting brain abnormalities under genetic control. However, the advantage of including only parents is that parents do not have a shared (pre- and perinatal) environment with the probands as do siblings [Sullivan et al. 2003]. Thus, if brain abnormalities were to be found in parents, they are more likely to be of heritable origin and consequently reflect endophenotypes of the disorder.

Conclusions

Biological, non-affected parents of autistic probands do not show brain enlargements. As the intracranium is not enlarged, it is unlikely that the brain volumes of the parents with an autistic proband have originally been enlarged and have been normalized. Thus, increased brain volumes in autism might be caused by the interaction of paternal and maternal genes, possibly with an additional effect of environmental factors, or increased brain volumes might reflect phenotypes of autism. Future twin and sibling studies may further enhance our understanding of the relative contributions of genes and environmental factors to the brain volume increases found in autism.

Declaration of Interest

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Chapter 7

Neuropathologic findings in autism

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Abstract

Autism is currently viewed as a largely genetically determined neurodevelopmental disorder, although its underlying biological causes remain to be established. In this review, we examine the available neuropathological literature on autism and discuss the findings that have emerged. Classic neuropathological observations are rather consistent in respect to the limbic system (9 of 14 studied cases showed increased cell packing density and smaller neuronal size), the cerebellum (21 of 29 studied cases showed a decreased number of Purkinje cells, and in all of 5 cases that were examined for age-related morphologic alterations, these changes were found in cerebellar nuclei and inferior olive), and the cerebral cortex (more than 50% of the studied cases showed features of cortical dysgenesis). However, all reported studies had to contend with the problem of small sample sizes, the use of quantification techniques not free of bias and assumptions, and high percentages of autistic subjects with comorbid mental retardation (at least 70%) or epilepsy (at least 40%). Furthermore, data from the limbic system and on age-related changes lack replication by independent groups. It is anticipated that future neuropathological studies hold great promise, especially as new techniques such as design-based stereology and gene expression are increasingly implemented and combined, larger samples are analyzed, and younger subjects free of comorbidities are investigated.

Introduction

Autism is an oligogenic neurodevelopmental disorder with a heritability of more than 90% [Bailey et al. 1996]. It is defined by the presence of marked social deficits, specific language abnormalities, and stereotyped, repetitive behaviors [American Psychiatric Association 1994]. Kanner, the first to report on autism, noticed the presence of enlarged heads in some of the children with autism [Kanner 1943]. Several subsequent studies have replicated this finding. Macrocephaly, defined as head circumference above the 97th percentile, was found in ~20% of subjects with autism [Aylward et al. 2002;Bailey et al. 1993;Courchesne et al. 2003;Davidovitch et al. 1996;Fidler et al. 2000; Fombonne 2000;Lainhart et al. 1997;Miles et al. 2000;Naqvi et al. 2000; Stevenson et al. 1997;van Karnebeek et al. 2002;Woodhouse et al. 1996]. Macrocephaly is not present until the first year of life, however [Courchesne et al. 2003;Lainhart et al. 1997;Stevenson et al. 1997]. Consistent with these clinical findings, neuropathological studies have reported increased brain weight in autistic individuals [Bailey et al. 1998;Casanova et al. 2002a;Courchesne et al. 1999;Kemper and Bauman 1998]. Likewise, neuroimaging studies have shown increased brain size in autistic children [Aylward et al. 2002;Carper et al. 2002;Courchesne et al. 2001;Filipek et al. 1992;Herbert et al. 2003;Sparks et al. 2002]. However, in autistic adolescents and adults, compared to control subjects, contradictory results have been reported either of increased brain volume [Hardan et al. 2001a;Hardan et al. 2001b;Piven et al. 1995;Piven et al. 1996], or of no difference in brain volume [Aylward et al. 1999;Aylward et al. 2002;Carper et al. 2002;Courchesne et al. 2001;Haznedar et al. 2000;Rojas et al. 2002;Townsend et al. 2001].

Thus, although abundant evidence of increased head circumference, brain weight, and brain volume in autism -especially in children- exists, the underlying biologic mechanisms of brain enlargement remain to be determined and could involve increased neurogenesis, increased gliogenesis, increased synaptogenesis, disturbed migration of neurons, decreased apoptosis, decreased pruning, or complex combinations of these events. This review discusses the existing neuropathological literature on autism (see table 1) and elaborates on the possibilities for future research, especially in the fields of genetics and neuropathology.

Neuropathalogical alterations in distinct brain regions

Alterations in the limbic system

Bauman and Kemper were the first to investigate the limbic system in autistic cases. Several case-reports [Bauman and Kemper 1985;Bauman and Kemper 1987;Bauman and Kemper 1990] and an earlier review [Bauman 1991] were brought together in one final review by these authors [Kemper and Bauman 1993]. By surveying whole brain serial sections, 6 autistic cases (5 males, 5 with mental retardation and 4 with epilepsy, 9, 10, 12, 22, 28, and 29 years old) were compared to 6 age- and sex-matched controls. All autistic cases showed increased cell packing density and reduced cell size in hippocampus, subiculum, and amygdala, and, although to a lesser extent, in entorhinal cortex, mammillary bodies, and septal nuclei. This pattern of small, closely packed neurons, with limited dendritic arbors, resembles that typically seen during earlier stages of brain maturation and may, therefore, reflect features of an immature brain [Jacobson 1991;Leroy Conel 1939]. A case report of a 16-year-old female with autism and severe mental retardation showed macroscopically low brain weight (1000 g), ventricular dilation, and a thin corpus callosum [Guerin et al. 1996]. Microscopically, however, no abnormalities were observed in the limbic structures, the cerebral cortex, and the cerebellum. Based on an earlier hypothesis by Lyon (1990), Guerin et al. (1996) proposed that these findings indicate a reduced density of axons and dendrites in the autistic brain. However, this hypothesis has not been tested in a larger sample size of autistics thus far. Using the Golgi method, the hippocampus of two autistic subjects (a 7-year-old female and a 9-year-old male, both with mental retardation, but without epilepsy) and two control subjects (8 and 13 years old) was examined [Raymond et al. 1996]. Only one autistic case had adequate CA4 neuronal staining, showing smaller neurons in the CA4 field compared to an age-matched control case. Both autistic cases showed less extensive dendritic branching in the CA1 and CA4 fields. These findings of reduced cell size and simplified dendritic pattern, without dysmorphic features, were consistent with a curtailment of maturation, as suggested previously by Kemper and Bauman [Kemper and Bauman 1993]. Bailey and colleagues investigated 6 autistic cases (all with mental retardation and three with epilepsy) and 7 age- and sex-matched controls [Bailey et al. 1998]. In only one of the 5 examined cases, increased cell packing density was observed in all CA subfields of the hippocampus.

Table 1: Neuropathologic findings in autism

	Author and year	Journal	Sample size & characteristics	Region of interest	Results
1	Williams et al. 1980	Arch Neurol	**4A** 3m; 4,14,27,33y; 4MR; 2E	Whole brain	Nerve cell loss and replacement gliosis in atrophic orbitofrontal and temporal regions in cases 1 and 3; ↓ Purkinje cell density in case 1
2	Bauman & Kemper 1985	Neurology	**1A** 1m; 29y; 1MR; 1E 1C; 1m; 25y	Whole brain	↑ cell-packing density and ↓ cell size in HIP, subiculum, entorhinal cortex, septal nuclei, mammillary body and selected nuclei of the AMY. Atrophy of neocerebellar cortex, with marked ↓ of Purkinje cells and cerebellar nuclei contained ↓ numbers of neurons, with the remaining neurons being small and pale.
3	Coleman et al. 1985	J Aut Dev Disord	**1A** 0m; 21y; 1MR; 0E 2C; 0m; 18 and 25y	Auditory cortex and Broca's area	No differences, except for ↓ glia in left auditory cortex and ↓ numbers of pyramidal neurons in right auditory association cortex
4	Ritvo et al. 1986	Am J Psychiatry	**4A** 4m; 10, 19, 19, 22y; 3MR; 0E 3C; 3m; 3, 10, 13y	CB	↓ Purkinje cell counts in both CB hemisphere and vermis
5	Bauman & Kemper 1987	Neurology	**1A** 0m; 11y; ?MR; 0E 2C; ?M; age-matched	AMY and HIP	↑ cell-packing density in HIP and AMY
6	Bauman & Kemper 1990	Neurology	**1A** 1m; 12y; 0MR; ?E 2C; 2m; age-matched	Limbic system and CB	↑ cell packing density of smaller neurons in limbic system; ↓ numbers of Purkinje cells; enlarged neurons in deep cerebellar nuclei and inferior olive

7	Bauman 1991	Pediatrics	**5A** 4m; 9 (new), 11, 22 (new), 28 (new), 29y (including study numbers 2 and 5 from the table); 4MR; 4E (3 new)	Limbic system in 4 cases and CB in all 5 cases	Review of earlier findings of ↑ cell packing density in limbic system (4/5) and ↓ Purkinje cell numbers in CB (5/5)
8	Hof et al. 1991	Acta Neuopathol (Berl)	**1A** 0m;24y;1MR;0E	Detection of neurofibrillary tangles in cerebral cortex and limbic system	Microcephaly (773 g) Neurofibrillary tangles, especially in layer II and III of temporal cortex, probably due to head banging
9	Fehlow et al. 1993	Pediatr Grenzgeb	**1A** 1m; 19y; 1MR; 0E	CB	Purkinje cell loss in lobules VI and VII
10	Kemper & Bauman 1993	Neurologic Clinics	**6A** (including all subjects of study number 7 from the table) 5m; 9,10,12 (new),22,28,29y; 5MR (1 new); 4E (0 new) 6C; age- and sex-matched	Limbic system and CB	Small and densely packed neurons in limbic system (6/6); ACC coarse and poorly laminated in 5/6; ↓ Purkinje cell numbers in CB (6/6)
11	Guerin et al. 1996	Dev Med Child Neurol	**1A** 0m; 16y; 1MR; 1E	Whole brain	Macroscopic: microcephaly, ↑ VENT; thin CC Microscopic: no abnormalities
12	Raymond et al. 1996	Acta Neuropathol	**2A** 1m; 7 and 9y; 2MR; 0E 2C; ?M; 8 and 13y	HIP	Smaller neurons in CA4; less dendritic branching in CA1 and CA4
13	Rodier et al. 1996	J Comp Neurol	**1A** 0m; 21y; 1MR; 1E 1C; 1m; 80y	Pons, medulla, and CB	Near-complete absence of the facial nucleus and superior olive along with shortening of the brainstem
14	Bailey et al. 1998	Brain	**6A** 6m; 4 and 20-27y; 6MR; 3E 7C; 5m; age-matched	Whole brain with neuronal counts in SFG, CB, HIP	Megaencephaly in 4; abnormalities inferior olives in 4; ↓ Purkinje cells in all adults; cortical dysgenesis in least 50%
15	Blatt et al. 2001	J Aut Dev Disord	**4A** 4m;19,19,20,22y; 4MR; 2E 3C; 3m; 16,19,24y	GABAergic, serotonergic, cholinergic, glutamatergic system in HIP	↓ GABAergic receptor system

16	Fatemi et al. 2001	Synapse	**5A** 5m; 22y; at least 3MR; ?E 4C; 4m; 24y	Bcl-2 and p53 in parietal cortex	32% ↓ Bcl-2; 130% ↑ p53
17	Fatemi et al. 2001	Neuroreport	**5A** 5m; 25y; ?MR; ?E 8C; 8m; 24y	CB cortex	34-51% ↓ Bcl-2
18	Fatemi et al. 2001	J Aut Dev Disord	**5A** (same subjects as in study number 17 from the table) 5m; 25y; ?MR; ?E 8C; 8m; 24y	Reelin and Bcl-2 in CB cortex	>40% ↓ Reelin and 34-51% ↓ Bcl-2
19	Perry et al. 2001	Am J Psychiatry	**7A** 6m; 24y; probably 7MR; at least 50%E 10C; 8m; 32y 9MR; 5m; 32y	Frontal and parietal cortex and basal forebrain	30% ↓ M1 receptor binding in parietal cortex; 65-73% ↓ α4 nicotinic receptor binding in frontal and parietal cortex; ↑ BDNF in forebrain
20	Casanova et al. 2002	J Child Neurol	**2AS** 2m; 22 and 79y; 0MR; ?E 18C; 18m; 9-98y	Layer III of prefrontal and temporal cortex	Cell columns were more numerous, smaller, and less compact
21	Casanova et al. 2002	Neurology	**9A** 7m; 12y; 7MR; 5E 9C; ?M; 15y	Layer III of prefrontal and temporal cortex	Cell columns were more numerous, smaller, and less compact
22	Fatemi et al. 2002b	Biol Psychiatry	**5A** 8C (CB) 4C (parietal cortex) (same subjects as in study 16 and 17 from the table)	GAD 65 and 67 kDa proteins in CB and parietal cortex	↓ 65 kDa 48% in parietal cortex and 50% in CB; ↓ 67 kDa 61% in parietal cortex and 51% in CB
23	Fatemi et al. 2002a	Cell Mol Neurobiol	**5A** (same subjects as in study number 17 from the table) 5m; 25y; ?MR; ?E 5C; at least 4m; 24y	CB	24% smaller Purkinje cells; no differences in density

24	Lee et al. 2002	Brain	**8A** (7 overlapping with study 19 from the table) 7m; 25y; 7MR; 5E 10C; 6m; 28y 11MR; 7m; 33y	CB	↓ α3 and α4 nicotinic receptor binding in granule cell, Purkinje and molecular layers; ↑ α7 nicotinic receptor binding in granule cell layer
25	Araghi-Niknam & Fatemi 2003	Cell Mol Neurobiol	**5A** 5m; 24y; ?MR; ?E 4C; 4m; 24y	Cerebellar and superior frontal cortex	↓ Bcl-2 and ↑ p53 both in cerebellar (36% resp. 38%) and superior frontal cortex (38% resp. 68%)

Abbreviations in alphabetical order: A, autistic subjects; ACC, anterior cingulate cortex; AMY, amygdala; AS, subjects with Asperger's syndrome; BDNF, brain-derived neurotrophic factor; C, control subjects; CB, cerebellum; CC, corpus callosum; E, epilepsy; GAD, glutamic acid decarboxylase; HIP, hippocampus; m, male; MR, mental retardation; SFG, superior frontal gyrus; VENT, ventricles; y, years of age. ↓, decreased; ↑, increased; ?, not mentioned.
Example of how to read the column "sample sizes and characteristics": In the first study [Williams et al. 1980] four autistic subjects were studied, three of them were male, ages were 4, 14, 27, and 33 years (either separate ages, or mean age, or age range is mentioned, dependent on the information given in the article), all four were mentally retarded, two had epilepsy as well. No controls were included.

Alterations in the cerebellum

Williams and colleagues were the first to perform a detailed neuropathological analysis on 4 individuals with autistic behavior (3 males, 12, 27, and 33 years of age and one female, 3 years of age, all presenting with mental retardation and 2 with seizures) [Williams et al. 1980]. Cortical and subcortical structures and the cerebellum were examined. Nerve cell loss and replacement gliosis were found in atrophic orbitofrontal and temporal regions in two cases, which were probably due to cerebral trauma that occurred some time after the development of autistic symptoms. The only abnormality, likely to be associated with autism was reduced Purkinje cell density in one case, with concomitant epilepsy and profound mental retardation. Thus, no clues as to the cause or the anatomic-pathologic substrate of autistic behavior could be obtained from these cases. Ritvo and colleagues counted Purkinje cells in the cerebellum of 4 autistic cases (all males, 3 with mental retardation, none with seizures) and 3 male controls [Ritvo et al. 1986]. Autistic cases showed a decreased number of Purkinje cells in the cerebellar hemisphere and vermis. Apart from reports on limbic alterations in 6 autistic cases, Kemper and Bauman reported on alterations in the

cerebellum as well [Kemper and Bauman 1993]. All 6 autistic cases showed decreased numbers of Purkinje cells (see also figure 1). As there was no evidence of glial cell hyperplasia or of retrograde olivary cell loss - both characteristic of a postnatal cerebellar insult [Holms and Stewart 1908;Rakic and Sidman 1970] - a lesion acquired early in development was suggested. Furthermore, in the two young autistic cases, the neurons in the deep cerebellar nuclei and the inferior olive were large, whereas in the autistic cases, older than 22 years, these neurons were small and pale. It should be mentioned that in normal development, projections from the inferior olive to the Purkinje cells are preceded by projections from the inferior olive to the cerebellar nuclei [Flechsig 1920]. Accordingly, a decreased number of Purkinje cells (which are the final target of the inferior olive projections) may result in an abnormal development of these fetal projections from the inferior olive to the cerebellar nuclei. However, as this fetal circuit was meant to function only for a short period of time, it was postulated that this circuit would eventually fail, resulting in the atrophy of the involved cells. A case report documented a 19-year-old man, presenting with Ehlers-Danlos syndrome and concomitant mental retardation and autism, who died of a mechanical ileus due to excessive aerophagia [Fehlow et al. 1993]. This case also exhibited a marked decrease in the number of Purkinje cells in cerebellar lobules VI and VII. However, another case report, of a 16-year-old female with autism and severe mental retardation [Guerin et al. 1996], showed no abnormalities in the cerebellum. Bailey and colleagues, in their study of 6 autistic cases (all with mental retardation and three with epilepsy) and 7 age- and sex-matched controls, reported low Purkinje cell counts in all 5 adult autistic cases, but not in the cerebellum of the 4-year-old autistic boy [Bailey et al. 1998]. Harding and Copp (1997) stated that, considering the normal development of the cerebellar cortex, it would be unlikely that the reported decreased number of Purkinje cells occurred only before 30 weeks of gestation, as was suggested by Kemper and Bauman [Kemper and Bauman 1993]. A substantially decreased number of Purkinje cells before 32 weeks of gestation would be associated with hypoplastic folia [Harding and Copp 1997], which was not the case in the brains investigated by Bailey and colleagues. In addition, the reported modest glial hyperplasia would have been another indication of a postnatal decrease in the number of Purkinje cells. Lee and colleagues examined two autistic cases (both with mental retardation, one with epilepsy) and observed a decreased number of Purkinje cells in both cases, whereas cerebellar white matter thinning and demyelination was found in one case [Lee et al. 2002]. As a result of the consistently reported decreased numbers of Purkinje cells in autism, Fatemi and colleagues were the first to examine the size of the cerebellar Purkinje cells [Fatemi et al. 2002a]. Blocks of the cerebella of five adult male autistic subjects (same subjects as [Fatemi et al. 2001a]) were compared to those of five age- and sex-matched controls. A 24% decrease in mean Purkinje cell size was found in the autistic group.

Alterations in the brainstem

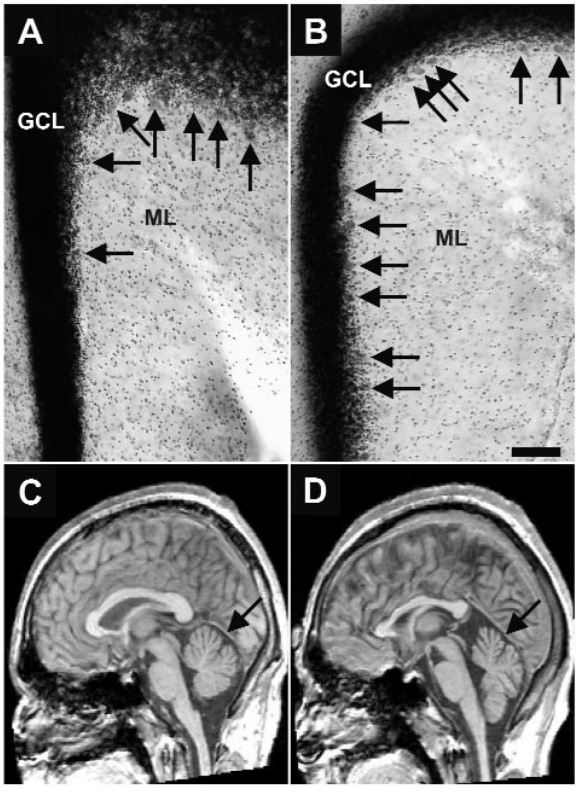
As already mentioned in the previous section, Kemper and Bauman reported alterations in the inferior olive [Kemper and Bauman 1993]. In the three young autistic cases (9, 10, and 12 years of age), the neurons in the inferior olive were large, whereas in the autistic cases (older than 22 years) these neurons were small and pale, but adequate in number. Furthermore, in all 6 autistic cases, some of the olivary neurons tended to cluster at the periphery of the nuclear complex. Although the significance of these findings remains to be elucidated, this pattern has been described earlier in some syndromes of prenatal origin that are associated with mental retardation [Sumi 1970;DeBassio et al. 1985]. The brainstem of a 21-year-old autistic woman with mental retardation and epilepsy [Rodier et al. 1996], showed a near-complete absence of the facial nucleus and superior olive along with shortening of the brainstem between the trapezoid body and the inferior olive, when compared to an 80-year-old male control case. Bailey and colleagues reported olivary dysplasia in 3 of the 5 autistic cases, as well as ectopic neurons related to the olivary complex in another two cases [Bailey et al. 1998].

Alterations in the neocortex

By counting pyramidal cells, other neurons, and glial cells in the primary auditory cortex, Broca′s language area, and the auditory association cortex, Coleman and colleagues failed to find consistent differences between the brain of a 21-year-old autistic female, with probable mental retardation, but without seizure disorder, and two control brains (both from females, 18 and 25 year old respectively) [Coleman et al. 1985]. The differences between the two control cases were larger than those between both of them and the autistic case. Of the 42 comparisons made, only 6 revealed differences between the autistic case and the control cases (i.e., decreased number of glial cells in the left primary auditory cortex and decreased number of pyramidal neurons in the right auditory association cortex in the autistic case), whereas 20 comparisons showed differences between the two control cases. Numerous neurofibrillary tangles were found in layer II and III of the cerebral cortex –especially in the temporal region- of a 24-year-old woman with autism and mental retardation, and severe self-injury behavior [Hof et al. 1991]. A few neurofibrillary tangles were found in the amygdala as well. It was suggested that these neurofibrillary tangles may be related to severe and chronic head injury, similar to boxers′ encephalopathy, where such abnormalities have been observed as well [Corsellis et al. 1973;Hof et al. 1992]. Kemper and Bauman, investigating 6 autistic cases, reported an unusually coarse and poorly laminated anterior cingulate cortex in 5 of the 6 autistic cases [Kemper and Bauman 1993]. A 16-year-old female with autism and severe mental retardation showed no abnormalities in the cerebral cortex [Guerin et al. 1996]. Bailey and colleagues reported no alterations in neuronal counts of the superior frontal cortex of the 6 autistic cases compared to 7 age-matched control cases [Bailey et al. 1998].

Figure 1

Panels A and B show representative photomicrographs from 200 μm thick frontal sections of post mortem brains from a 13-year-old male suffering from autism (Panel A) and from a 14-year-old male control (Panel B). These pictures show a part of the cerebellum (GCL, granule cell layer; ML, molecular layer). Note the smaller number of Purkinje cells in the brain from the autistic patient compared to the control (arrows). These photomicrographs were produced using a video camera (Hitachi HV-C20A; Hitachi, Japan) attached to an Olympus BX 50 microscope and the Stereo Investigator software (microbrightfield, Williston, VT). Twelve separate images were needed each to cover the parts of the cerebellum shown. For each separate image the microscope was focused on the Purkinje cell layer. These images were then assembled into one montage using the Virtual Slice module of the Stereo Investigator software. Final images were constructed using Corel Draw v. 11. Only minor adjustments of contrast and brightness were made, which in no case altered the appearance of the original materials. Bar, 400 μm. Furthermore, Panels C and D show representative MRI scans of the cerebellar midsagittal areas from a 16-year-old male suffering from autism (Panel C) and from a 16-year-old male control person (Panel D) (arrows). Note the somewhat smaller cerebellar midsagittal area in the brain from the autistic patient compared to the control.



Observations on cortical dysgenesis and migration abnormalities

Bailey and colleagues reported cortical dysgenesis in 4 of 6 autistic cases, with thickened cortices, high neuronal density, presence of neurons in the molecular layer, irregular laminar patterns, and poor gray-white matter boundaries [Bailey et al. 1998]. White matter abnormalities were found in 4 cases as well, including ectopic gray matter in three cases and increased number of white matter neurons in one case. The authors stated that cerebral developmental abnormalities, such as megaencephaly (which was present in 4 of the 6 cases), are usually associated with heterotopias [Harding and Copp 1997], that were also found in the present cases. Fatemi and colleagues pursued the investigation of the neurochemical parallels of decreased Purkinje cell counts that have been consistently reported in autism. In two overlapping studies, levels of reelin [Fatemi et al. 2001a] and Bcl-2 [Fatemi et al. 2001b;Fatemi et al. 2001a] were measured in the cerebellar cortex of 5 adult autistic males (IQ and seizure status unknown) and 8 adult controls. Reelin, the product of the reelin gene, is a signaling protein that is involved in the control of neuronal migration and correct lamination during the embryonic period, and of synaptic plasticity in adult life [Fatemi 2002]. The Bcl-2 protein governs programmed cell death (apoptosis) in the developing brain. More than 40% reduction in reelin and 34%-51% reduction in Bcl-2 were found by Fatemi and colleagues [Fatemi et al. 2001b;Fatemi et al. 2001a]. Reduction in reelin has been found to be associated with disturbed neuronal migration and lamination of the cerebral and cerebellar cortex in mice [Fatemi 2001;Fatemi et al. 1999;Gonzalez et al. 1997] and was suggested to be involved in migrational processes during the early development of the human brain [Persico et al. 2001;Piven et al. 1990]. Moreover, reductions in blood reelin have been associated with severe mental retardation and hypoplastic cerebellum, findings that have both been reported in autism. The reported reduction in Bcl-2 might influence programmed cell death as this protein strongly inhibits apoptosis. Following these reports of reduced levels of the anti-apoptotic protein Bcl-2 in the cerebellum from autistic patients [Fatemi et al. 2001b;Fatemi et al. 2001a], levels of Bcl-2 and p53 (a key regulator of neuronal apoptosis [Araki et al. 2000]) were measured in the parietal and superior frontal cortex of five adult autistic males (three with mental retardation, seizures unknown) and four adult male controls [Araghi-Niknam and Fatemi 2003;Fatemi and Halt 2001]. A reduction of more than 30% (32% in parietal and 38% in superior frontal cortex) in Bcl-2 expression was reported, comparable to the reduction observed in the cerebellar cortex [Fatemi et al. 2001b;Fatemi et al. 2001a]. In contrast, an increase in p53 expression (130% in parietal and 68% in superior frontal cortex) was found. These abnormalities in Bcl-2 and p53 were correlated with the presence of severe mental retardation (mean IQ of the patients was 25). Both the decrease in the anti-apoptotic Bcl-2 and the increase in apoptosis-controlling p53 were thought to result in a greater propensity for cell death. Indeed, it was suggested previously that

increased brain volume in autism may be found only in high-functioning subjects [Akshoomoff et al. 2002], whereas autistic subjects with (severe) mental retardation would display normal or even smaller brain volumes compared to controls.
Recently, the configuration of so-called minicolumns was investigated in autism and Asperger syndrome [Casanova et al. 2002b;Casanova et al. 2002c]. Casanova and colleagues posed that cell minicolumns are supposed to be a basic functional unit of the brain that organizes neurons in cortical space [Mountcastle 1997]. Instead of cell counting, an overall cell density measure was used, estimating the amount of space occupied by cell somas in a certain predefined area. More numerous, smaller and less compact minicolumns were found in nine autistic subjects (7 with mental retardation, 5 with epilepsy, 4 with macroencephaly) compared to 9 control cases [Casanova et al. 2002b] and in two adults with Asperger's syndrome compared to 18 control subjects [Casanova et al. 2002c]. However, the functional significance of these minicolumns is still unclear [Hutsler and Galuske 2003]. Several attempts have been made to identify these minicolumns as the anatomical correlate of the smallest processing unit in the cerebral cortex; however, further research will be required to solve unequivocally this issue [Jones 2000].

Alterations in the cholinergic system

The cholinergic system has been shown to play a significant role in cortical development [Hohmann and Berger-Sweeney 1998]. Cholinergic afferents innervate the cerebral cortex during the most dynamic periods of neuronal differentiation and synapse formation. Disruption of cholinergic innervation during early postnatal development results in delayed cortical neuronal development and permanent changes in cortical architecture and cognitive function [Hohmann and Berger-Sweeney 1998]. Abnormalities have been found in the basal forebrain (septal) cholinergic neurons of autistic cases, such as larger neurons at younger ages and smaller neurons at older age [Bauman and Kemper 1994]. Perry and colleagues investigated cholinergic biomarkers in the basal forebrain and the (frontal and parietal) cerebral cortex in the brains of 7 autistic cases (all with mental retardation, at least 50% with epilepsy), 9 mentally retarded but not autistic cases, and 10 controls [Perry et al. 2001]. In the autistic cases, muscarinic M1 receptor binding was found to be 30% lower in the parietal cortex compared to both the normal comparison cases and to the non-autistic mentally retarded cases. In addition, $\alpha 4$ nicotinic receptor binding was reduced by 65-73% in the frontal and parietal cortex in both autistic and non-autistic, mentally retarded cases compared to the controls. In the basal forebrain of autistic subjects, the only abnormality was an increase in brain-derived neurotrophic factor (BDNF), an increase that had been previously found in neonatal bloodspots of children who later developed autism or mental retardation [Nelson et al. 2001]. These results indicated normal presynaptic cholinergic activity, but abnormal postsynaptic cholinoceptive function, the M1 receptor being located postsynaptically.

Following the report of Perry and colleagues (2001), the same group examined cholinergic activities in the cerebellum of these autistic cases (with an additional one). Eight autistic adults (7 with mental retardation, 5 with epilepsy), 11 age-matched subjects with mental retardation but no autism, and 10 age-matched controls were included in this study [Lee et al. 2002]. In the autistic cases, the nicotinic receptor, consisting primarily of α3 and α4 subunits, was reduced by 40-50%, whereas an opposite increase in nicotinic receptor, consisting of the α7 subunit, was reported. The exact relationship between these receptor abnormalities and autism and mental retardation remains to be determined.

Alterations in the GABAergic system

Like the cholinergic system, the GABAergic system has an important role in early neuronal development as well, and has been suggested to be involved in autism as well [Cook et al. 1998;Schroer et al. 1998]. During the early neonatal period, GABA provides most of the excitatory drive to developing neurons rather than being an inhibitory neurotransmitter [Barker et al. 1998;Cherubini et al. 1991]. Blatt and colleagues (2001) investigated 4 neurotransmitter systems (i.e., the GABAergic, serotonergic, cholinergic, and glutamatergic system) in the hippocampus of 4 autistic adult male cases (all with mental retardation and 2 with epilepsy) and 3 adult male control cases [Blatt et al. 2001]. The GABAergic system was the only neurotransmitter system found to be significantly reduced in autism. The other three neurotransmitter systems did not show any differences between the autistic and control cases. Although the significance of these findings is not clear, it was suggested that a decrease in the availability of inhibitory GABA receptors could alter receptor activity. As a consequence, the threshold for development of seizures, a frequent comorbidity of autism [Bailey et al. 1998], would be reduced. Fatemi and colleagues investigated the level of glutamic acid decarboxylase (GAD), the rate limiting enzyme responsible for the conversion of glutamate to GABA in the brain [Fatemi et al. 2002b]. The levels of the 65 kDa and the 67 kDa GAD were measured in the cerebellum of 5 autistic and 8 control cases and in the parietal cortex of 5 autistic cases (3 overlapping with the cerebellum cases) and 4 control cases (all overlapping with the cerebellum cases). The 65 kDa GAD protein was reduced by 50% in the cerebellum and by 48% in the parietal cortex of the autistic cases. The 67 kDa GAD protein was reduced by 51% in the cerebellum and by 61% in the parietal cortex of the autistic cases. These decreases in GAD were thought to subserve a deficit in GABA availability, as reported by Blatt and colleagues [Blatt et al. 2001]. In addition, a deficit in GABA, was not only suggested to play a role in the etiology of seizures: it was also proposed to affect several important biological functions, such as locomotor activity, learning, and circadian rhythms [Soghomonian and Martin 1998]. However, as the sample sizes and the number of studies on the GABAergic system in autism have been thus far very small, no definite statement can be made about the exact role of the GABAergic system in the etiology of autism.

Are MRI findings in autism a structural observable correlate to the neuropathological findings?

Research on the neuropathology of autism has been hampered by the lack of availability of large sample sizes and closely matched control groups. Structural MRI, on the other hand, is uniquely suited to scan (repeatedly) the brains of large groups of (young) patients and matched controls in vivo and map neuroanatomic abnormalities. Unfortunately, to date structural MRI findings cannot be directly correlated to the neuropathological findings in autism, although the repeatedly reported increased brain volume detected with MRI [Aylward et al. 2002;Carper et al. 2002;Courchesne et al. 2001;Filipek et al. 1992;Hardan et al. 2001a;Hardan et al. 2001b;Herbert et al. 2003;Piven et al. 1995;Piven et al. 1996;Sparks et al. 2002] seems consistent with the frequent observation of an increased brain weight in autism [Bailey et al. 1998] (see also [Courchesne et al. 2000;Casanova et al. 2002a;Courchesne et al. 1999;Kemper and Bauman 1998]). Neuropathological studies have consistently reported smaller and more closely packed neurons in the limbic system in autistic patients, whereas MRI findings are rather equivocal. Volumes of limbic structures of autistic subjects have been found either increased [Howard et al. 2000;Sparks et al. 2002], decreased [Aylward et al. 1999;Herbert et al. 2003;Pierce et al. 2001;Saitoh et al. 2001], or unchanged [Haznedar et al. 2000;Howard et al. 2000;Piven et al. 1998;Saitoh et al. 1995] compared to those of control subjects. Likewise, the consistent observation of a decrease in Purkinje cell number and density does not have an MRI equivalent. Although early MRI reports consistently showed smaller midsagittal cerebellar hemispheres [Gaffney et al. 1987;Murakami et al. 1989] or vermis [Ciesielski et al. 1997;Courchesne et al. 1988;Hashimoto et al. 1995] in autism (see also figure 1), more recent reports did not [Filipek et al. 1992;Garber and Ritvo 1992;Holttum et al. 1992;Kleinmand et al. 1992;Nowell et al. 1990;Piven et al. 1992;Piven et al. 1997]. This lack of agreement in cerebellar segmentation between neuroimaging studies, might be partially explained by using different MRI systems, as was most recently reported [Lotspeich et al. 2004]. It is important to keep in mind that generally these studies have not accounted for IQ as a confounding factor [Piven et al. 1992]. Thus, although both neuropathological and MRI studies investigate brain structures, the two techniques have failed to provide correlated and consistent data.

Discussion

In this review we have attempted to provide an extensive overview of the available neuropathological literature of autism. Although some consistent results emerge, the majority of the neuropathological data remain equivocal. This may be due to lack of statistical power, resulting from small sample sizes and from the heterogeneity of the disorder itself, to the inability to control for potential confounding variables such as gender, mental retardation, epilepsy, and medication status, and importantly, to the lack of consistent design in histopathological quantitative studies of autism published to date. Furthermore, the investigation of different brain structures could have contributed to the disparate findings. However, when considering the available data, a number of conclusions can be drawn (table 2). First, a decrease in the number of Purkinje cells throughout the cerebellar hemispheres without significant gliosis and features of cortical dysgenesis have been consistently found by different research groups. Second, although not replicated by independent research groups, increased cell packing density of smaller neurons in the limbic system and age-related abnormalities in the cerebellar nuclei and the inferior olive have been reported in the majority of the studied cases. Finally, both the nicotinic and muscarinic cholinergic and the GABAergic system are likely to be impaired in autism.

An arrest of normal development has been proposed to explain the findings in the limbic system, whereas the decrease in Purkinje cell numbers is likely to be largely prenatal in origin [Kemper and Bauman 1998]. The features of cortical dysgenesis, such as increased neuronal density, increased cortical thickness, ectopic gray matter, and poor differentiation of the gray-white boundary, are suggestive of abnormalities in cortical lamination (i.e., abnormalities in neuronal proliferation and migration) and apoptosis [Rorke 1994]. In support of this possibility are the findings of reductions in reelin (implicated in regulation of layering of the cortex) and Bcl-2 (implicated in the process of apoptosis).

As to the timing of the neuropathological abnormalities in autism, all authors have suggested a prenatal origin, most likely during the first 6 months of gestation [Bailey et al. 1998;Bauman et al. 1997;Courchesne 1997;Gillberg 1999;Piven et al. 1990;Rodier et al. 1996;Rorke 1994]. It should be mentioned however, that according to a hypothesis by Gillberg [Gillberg 1999], there could be at least two different pathways to autism, one connected with primary temporofrontal dysfunction (and late prenatal-early postnatal origins) and another linked to primary brainstem dysfunction (and early prenatal origins). Furthermore, a recent report by Kern has suggested that it is conceivable that some children become autistic from neuronal cell death or brain damage occurring postnatally as a result of injuries, as some cases of autism do not show symptoms until a substantial period after birth [Kinnear 2003].

Table 2: Consistent neuropathologic findings in autism

Finding	**N**	**N_new**	**MR**	**E**	**Results**
Increased cell packing density and smaller neurons in the limbic system					
Kemper & Bauman 1993	6	6	5	4	6 of 6
Guerin et al. 1996	1	1	1	1	0 of 1
Raymond et al. 1996	2	2	2	0	2 of 2 (only HIP measured)
Bailey et al. 1998	6	6	6	3	1 of 5 (only HIP measured)
Total	**15**	**15**	**14/15**	**8/15**	**9 of 14 (64%)**
Decreased numbers of/smaller Purkinje cells in CB					
Williams et al. 1980	4	4	4	2	1 of 4
Ritvo et al. 1986	4	4	3	0	4 of 4
Fehlow et al. 1993	1	1	1	0	1 of 1
Kemper & Bauman 1993	6		5	4	6 of 6
Guerin et al. 1996	1		1	1	0 of 1
Bailey et al. 1998	6		6	3	5 of 6 (only the child unaffected)
Fatemi et al. 2002a	5	5	?	?	2 of 5
Lee et al. 2002	2		2	1	2 of 2
Total	**29**	**14**	**22/24**	**11/24**	**21 of 29 (72%)**
Age changes in CB nuclei and inferior olive					
Bauman 1991	5		5	4	Large neurons in the 2 children, pale and small neurons in the 3 adults
Total	**5**	**0**	**5**	**4**	**5 of 5 (100%)**
Brainstem/olivary dysplasia					
Rodier et al. 1996	1	1	1	1	1 of 1
Bailey et al. 1998	5		5	3	3 of 5
Total	**6**	**1**	**6/6**	**4/6**	**4 of 6 (67%)**
Alterations in the neocortex					
Coleman et al. 1985	1	1	1	0	0 of 1
Hof et al. 1991	1	1	1	0	1 of 1 ↑ NFT

Kemper & Bauman 1993	6		5	4	5 of 6 poorly laminated ACC
Guerin et al. 1996	1		1	1	0 of 1
Bailey et al. 1998	6		6	3	0 of 6 abnormal FR neuronal count
Total	**15**	**2**	**14/15**	**8/15**	**6/15 (40%)**
Cortical dysgenesis					
Bailey et al. 1998	6		6	3	4 of 6
Fatemi et al. 2001	5	5	3 or >	?	As a group ↓ Bcl-2 and ↑p53 PA
Fatemi et al. 2001a	5		?	?	As a group ↓ Bcl-2 and Reelin CB
Casanova et al. 2002c	2AS	2AS	0	?	1 of 2 had smaller minicolumns
Casanova et al. 2002a	9	9	7	5	As a group smaller minicolumns
Araghi-Niknam & Fatemi 2003	5		?	?	As a group ↓ Bcl-2 and ↑p53 FR
Total	**32**	**14+2**	**16/22**	**8/15**	
Abnormalities in cholinergic system					
Perry et al. 2001	7	7	7	3 or >	As a group ↓M1 PA; ↓ α4 FR + PA; ↑BDNF forebrain
Lee et al. 2002	8	1	7	5	As a group ↓ α3 and α4 and ↑ α7 in CB
Total	**15**	**8**	**14/15**	**8/15**	
Abnormalities in GABAergic system					
Blatt et al. 2001	4	4	4	2	↓ GABAergic system in HIP
Fatemi et al. 2002b	5		?	?	↓ 65 and 67 kDa GAD in CB and parietal cortex
Total	**9**	**4**	**4/9**	**2/9**	

Abbreviations in alphabetical order: ACC, anterior cingulate cortex; AS, Asperger's syndrome; BDNF, brain-derived neurotrophic factor; CB, cerebellum; E, epilepsy; FR, frontal cortex; GAD, glutamic acid decarboxylase; HIP, hippocampus; MR, mental retardation; N, number of autistic subjects; N_new: number of brains mentioned for the first time (58 autistic and 2 Asperger); NFT, neurofibrillary tangles; PA, parietal cortex; ?, not mentioned

Indeed, Purkinje cells can be selectively vulnerable to certain types of insult such as ischemia, hypoxia, excitotoxicity, viral infections, heavy metals, and toxins such as ethanol [Welsh et al. 2002].

Taken together there is evidence from neuropathological data for an evolving pathologic process in the autistic brain that extends from the fetal period of brain development into adulthood. However, the mechanisms underlying these alterations remain unknown. Likewise, the underlying causes of the reported decreased nicotinic receptor binding in the cholinergic system is not understood, although it is known that nicotine enhances several cognitive and psychomotor behaviors [Granon et al. 2003], suggesting the potential for intervention through cholinergic receptor modulation. The same holds true for the apparent reduced function of the GABAergic system.

Thus, besides the ongoing classic neuropathological research, future studies will benefit from focusing on techniques aiming to disentangle the underlying biological mechanisms of autism. Design-based stereologic approaches to neuropathology may become a key methodology, as it can provide information about the degree of maturation or health of brain cells and overall brain development [Hof and Schmitz 2000;Schmitz and Hof 2004;West 1993]. Design-based stereology permits to measure precisely and reliably number, size, and spatial distribution of cells within a given brain region, using standardized protocols. Another relatively new approach, holding great promise in the immediate future of autism research, is the study of gene expression. It is expected that extensive and detailed investigations of gene expression will help to understand the molecular and cellular basis of the neuropathology of autism. Region-specific alterations in gene expression will be reflective of neuroadaptive processes underlying the neuropathological findings of autism reported in the literature. In the field of autism, gene expression is still in its infancy, although some results have already been published. For example, preliminary data have shown a complete absence of αB-crystallin, a small heat-shock protein functioning as a molecular chaperone, in the frontal cortex of brains from autistic patients [Pickett 2001]. Purcell and colleagues investigated the neural cell adhesion molecule (NCAM), a developmentally regulated protein, in a sample of 10 autistic cerebella and 16 control cerebella [Purcell et al. 2001a]. A decrease in the longest of three isoforms (180 kDa) of NCAM was found in the cerebella from the autistic patients. In addition, the mRNA levels of 2 genes, both members of the glutamate system were found to be increased in the cerebellum of the same autistic cases [Purcell et al. 2001b]. Identifying changes in gene expression will ultimately be useful to provide molecular diagnostic tests for autism and to identify specific cellular pathways that have been disrupted in this disorder.

In conclusion, classic neuropathological observations in autism show increased cell packing density and smaller neuronal size in the limbic system, decreased number of Purkinje cells in the cerebellum, and features of cortical dysgenesis

or migration disturbances. However, the underlying neurobiologic basis remains elusive. The implementation of newer techniques, such as design-based stereology and large-scale analysis of gene expression, holds great promise and might eventually result in the elucidation of the etiology of autism.

Note added in proof

A recent study applying functional MRI during sentence comprehension indicated a lower degree of information integration across large-scale cortical networks involved in language processing as a possible neural basis of disordered language in autism [Just et al. 2004]. It will be attractive to test this hypothesis in future neuropathological studies of autism.

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Chapter 8

Cortical neurons are more numerous in autism

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Submitted for publication

Abstract

The neuropathologic basis of autism remains elusive. Here we demonstrate the first evidence, using high-precision design-based stereology, of an increase in total neuron numbers in the cerebral cortex of autistic patients compared to age-matched controls. This finding represents a crucial step towards a better understanding of the pathophysiologic mechanisms underlying the possible abnormal development of neural systems in autism.

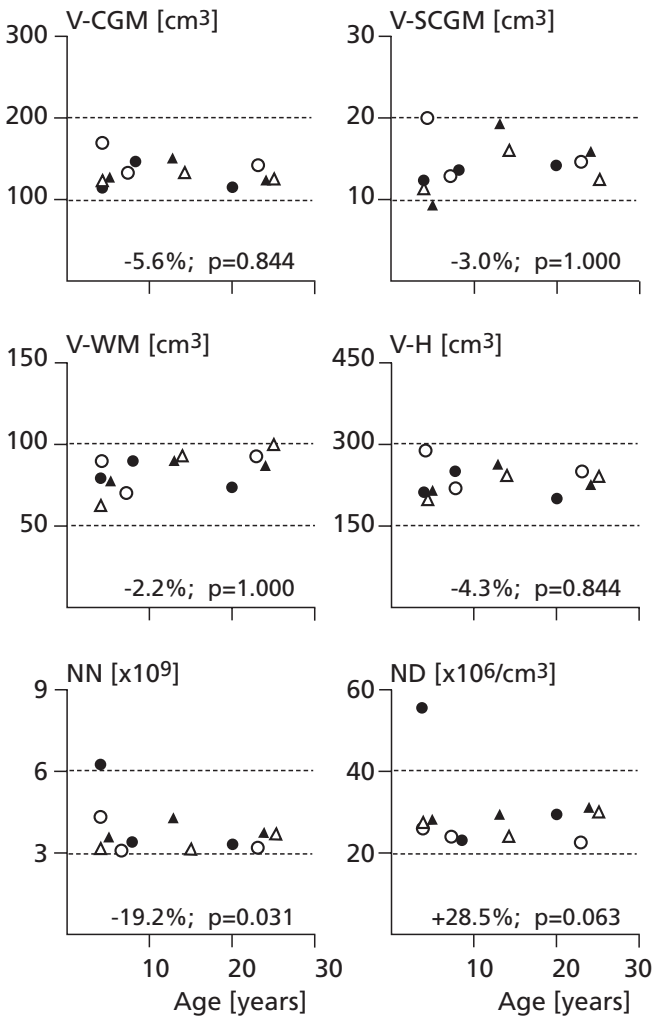
Autism is a neurodevelopmental disorder defined by the presence of social deficits, language abnormalities, and stereotyped behaviors [Rapin 1997]. Its underlying neuropathology is largely unknown [Palmen et al. 2004]. Following reports of increased head circumference [Courchesne et al. 2003], brain volume [Courchesne et al. 2001;Piven et al. 1995] and cell packing density [Kemper and Bauman 2002], we investigated whether autistic patients had abnormally high total neuron numbers in the cerebral cortex.

Whole postmortem hemispheres of 6 autistic patients (12.3 ± 3.4 years old) and 6 age-matched controls (12.8 ± 3.8 years old; table S1) were investigated with high-precision design-based stereology [Heinsen et al. 2000;Schmitz and Hof 2004; supporting online material]. The analysis showed very small differences in mean volumes of cortical gray matter, subcortical gray matter, white matter and whole hemisphere between autistic patients and controls (figure 1). In contrast, the autistic patients had significantly higher total neuron numbers in the cerebral cortex than the controls (on average +19%; $p = 0.031$; figure 1). The autistic patients also had higher overall cortical neuron densities than the controls (on average +29%; $p = 0.063$; figure 1), although this difference did not reach statistical significance due to high interindividual variability.

This study is the first to use rigorous quantitative histologic techniques to estimate total neuron numbers and densities in brains from autistic patients. The present stereologic data reveal important divergences from previous studies of autism pathology [Courchesne et al. 2001;Piven et al. 1995], as no increase in brain volume was observed. However, our findings support the notion of increased cell packing density in several forebrain areas, as well as the presence of widespread, rather than localized, brain alterations in autism [Akshoomoff et al. 2002;Eigsti et al. 2003;Kemper and Bauman 2002;Waiter et al. 2004]. Also, as the main effect was due to the difference within the youngest pair, our results point to an early insult, in agreement with previous reports [Courchesne et al. 2001;Courchesne et al. 2003]. In the context of brain development, it is worth noting that increased neuron density in the cerebral cortex, suggestive of increased total neuron numbers, has recently been identified in offspring of mice exposed to human influenza virus during pregnancy, a model considered one of the most promising animal approaches to autism [Fatemi et al. 2002;Shi et al. 2003].

Given the present evidence of higher cortical neuron numbers in autistic patients, it will become crucial to assess what areas of the cortex contribute most to the observed global difference and what neuronal cell types are preferentially affected. The histologic protocols used in the present study were designed to perform such investigations on the same brains [Heinsen et al. 2000]. Ultimately, studies combining rigorous quantitative histologic techniques with molecular analyses will be required to unravel the vulnerability profile of the neuronal populations that are specifically affected in autism.

Figure 1.
Cortical gray matter volume (V-CGM), subcortical gray matter volume (V-SCGM), white matter volume (V-WM), total hemispheric volume (V-H), total cortical neuronal number (NN) and overall cortical neuronal density (ND) from 6 autistic patients (black symbols) and 6 age-matched controls (open symbols) as a function of age. Individual data are shown as dots and diamonds to facilitate allocation of controls to the corresponding autistic patients. The difference in mean data is indicated (autistic patients vs controls) below each graph with the corresponding p values from Wilcoxon signed rank tests.



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Cortical neurons are more numerous in autism

by Saskia J.M.C. Palmen, Helmut Heinsen, Herman van Engeland, Harry W.M. Steinbusch, Patricia von Cappeln, Hubert Korr, Patrick R. Hof and Christoph Schmitz

Supporting Online Material

Brain specimens

Postmortem brains (one hemisphere per case) from 6 autistic cases (mean interval between death and autopsy 20.0 ± 2.9 hours) and from 6 age-matched controls (mean interval between death and autopsy 24.0 ± 11.1 hours) were analyzed. Clinical data are shown in table S1. Brains were obtained from several brain banks in the USA and Germany (see last reference in the main text). All autistic patients met the Diagnostic Statistical Manual, 4th revision (DSM-IV) and Autism Diagnostic Interview criteria of autism [Lord et al. 1994]. In all of the cases, autopsy was performed after informed consent was obtained from a relative. The use of these autopsy cases was approved by the relevant Institutional Review Boards. Clinical records were available for all cases.

Tissue processing

After immersion-fixation in 10% formalin for at least 3 months all hemispheres were mounted with celloidin and cut into entire series of 200 μm thick coronal sections as described elsewhere [Heinsen et al. 2000]. Three hemispheres were cut at 500 μm thickness. Every third (in one hemisphere: every second) section was stained with gallocyanin. These differences did not influence the results of this study.

Stereologic processing

On average 20 systematically and randomly sampled sections per hemisphere were analyzed with a stereology workstation (StereoInvestigator, MicroBrightField; Williston, VT, USA). Estimates of volumes of cortical gray matter (CGM), subcortical gray matter (SCGM) and white matter (WM) were carried out with Cavalieri's principle and point counting (see [Schmitz and Hof 2004] for review of all stereologic techniques applied). The counting procedure was implemented as follows: objective used for counting points: 1.25×; distance between points in mutually orthogonal directions x and y: 2,000 μm; measured actual average section thickness after histologic processing: 175 μm for hemispheres cut at 200 μm (465 μm for hemispheres cut at 500 μm, respectively); average number of counted points per case: 5,731 for CGM, 613 for SCGM and 3,607 for WM. Total hemispheric volumes were calculated as the sum of the

volumes of CGM, SCGM and WM.
Total neuronal numbers were investigated with the Optical Fractionator using the following design: objective used for counting neurons: 20×, oil, NA=0.8; base and height of the unbiased virtual counting spaces: 6,400 μm^2 and 20 μm, respectively; distance between the unbiased virtual counting frames in mutually orthogonal directions x and y: 6,500 μm; average sum of unbiased virtual counting frames used: 510; average number of counted neurons per case: 1,987. Measurements were done blind to diagnosis by S.J.M.C.P. until all hemispheres were analyzed and were independently crossevaluated by P.v.C.; the inter-rater variability was less than 5%.

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Table 1: Clinical characteristics of the cases included in this study

No	Sex	Site	Age [Y]	Cause of death	BW [g]	PMI [h]
A1	M	L	4	Drowning	1,160	30
C1	M	L	4	Myocardial infarct – Takayasu arteriitis	1,380	5
A2	F	L	5	Car accident	1,390	13
C2	F	R	4	Lymphocytic myocarditis	1,222	21
A3	M	R	8	Sarcoma	1,570	22
C3	F	R	7	Status asthmaticus	1,350	78
A4	M	L	13	Seizures	1,420	26
C4	M	R	14	Electrocution	1,600	20
A5	F	R	20	Obstructive pulmonary disease	1,108	15
C5	M	R	23	Ruptured spleen	1,520	6
A6	M	R	24	Drowning	1,610	14
C6	M	R	25	Cardiac tamponade	1,388	14

BW, brain weight; PMI, postmortem interval (hours); A, autism; C, control; M, male; F, female; L, left; R, right; Y, years.

Chapter 9

Structural brain abnormalities in autism: discussion

Autism is currently viewed as a largely genetically determined neurodevelopmental disorder. Over the last decades, an increasing number of studies have been performed, trying to establish the underlying biological causes of autism. However, its exact etiology still remains unclear. In this thesis, we have tried to list the existing literature on and enhance the knowledge of structural brain abnormalities as applied to autism. First, we have written two reviews, one on the existing structural MRI literature (chapter 2) and one on the available neuropathological literature (chapter 7). Second, we have performed 4 structural MRI studies, 3 investigating which brain structures are abnormal in volume in high-functioning medication-naive subjects with autism (chapter 3, 4, and 5) and one investigating to what extent the brain abnormalities, as found in autistic subjects, show genetic susceptibility (chapter 6). Third, using design-based stereology techniques, we have investigated brains of autistic and control subjects to explore the underlying cellular alterations in autism (chapter 8).

Before starting the neuroimaging studies, as described in the present thesis, we thought it necessary to sort out several substantial issues: what findings have been found consistently (and need not to be replicated anymore); what results are equivocal (and need more investigation); what issues are still unexplored (but need to be resolved); and what choices have been made in composing the samples. Therefore, in **chapter 2**, we reviewed the existing structural MRI literature as applied to autism. In short, increased brain volume is one of the most replicated findings in autism, although results are equivocal as to whether brain enlargement is a phenomenon restricted to early childhood or whether it is still present in older children, adolescents, and even adults. Two related, and up till now unanswered issues, are whether brain enlargement is confined to the gray and/or the white matter; and whether brain enlargement is global or more prominent in specific brain regions. Results on the cerebellum and the midsagittal corpus callosum seem to be unequivocal, i.e. increased respectively decreased in volume in autism. Finally, concerning the subcortical structures, no irrefutable evidence has been put forward, either for increases or decreases in volume. Thus, overall, results are rather inconclusive. This may be partly due to lack of statistical power in the face of the heterogeneity of the disorder itself, and partly to the failure to control for potential confounding variables, such as age, sex, IQ, socioeconomic status, and medication status. In order to elucidate (some of) the above-mentioned issues, we set out to investigate homogeneous groups of medication-naive high-functioning subjects with autism and closely matched control groups. Our first aim was to resolve as to what age brain enlargement is present in autism. Second we investigated whether gray or white matter volume is preferentially affected. Third, we investigated whether morphologic brain abnormalities in autism are widespread, or, on the other hand, rather localized.

In **chapter 3 and 4**, we demonstrated that both medication-naive, high-functioning children (chapter 3) and adolescents and young adults (chapter 4) with autism displayed a global increase of 5-6% in cortical gray –but not cerebral white- matter and cerebellar volume, an increase that was proportional to the increase in brain volume. In addition, a disproportional increase in ventricular volumes was found, which seemed to increase with age. Although it was suggested previously that increased brain volume might only be present in (young) children [Courchesne et al. 2001], we reasoned that, at least in high-functioning patients with autism, brain enlargement may still be present in adult life, a hypothesis that has been put forward earlier [Akshoomoff et al. 2002]. Indeed, as several studies in healthy subjects have revealed a significant positive correlation between brain volumes and intelligence measures (see e.g. [Posthuma et al. 2002;Thompson et al. 2001]) and mentally retarded individuals have shown on average a decrease in mean head circumference of two or more standard deviations compared to that of healthy individuals [Mosier, Jr. et al. 1965;Nellhaus 1968], it might very well be possible that increases in brain volume associated with autism are nullified by decreases in brain volume associated with mental retardation. With MRI, one can only speculate as to the nature of the underlying pathophysiology of the global gray matter volume enlargement in autism. Increased neurogenesis, increased gliogenesis, and decreased pruning and apoptosis have been suggested to occur in autism [Piven et al. 1996]. Interestingly, as serotonin is known to block apoptosis [Azmitia 2001], it is possible that the reported hyperserotonemia in autism [Mulder et al. 2004;Veenstra-VanderWeele et al. 2002] excessively blocks apoptosis. Surprisingly, the increase in both lateral and third ventricular volume was disproportionally large and increasing with age. One could speculate that subcortical structures in the neighborhood of the ventricles are smaller in autism, although supporting evidence for this theory is weak [Herbert et al. 2003;Tsatsanis et al. 2003]. Another possible explanation may be that the brain is enlarged more than 6% in patients younger than 7 years of age and that therefore brain volume decreases relatively more rapidly in patients compared to controls, resulting in disproportionally increased ventricular volume.

Following these reports of global cortical gray matter enlargement, we wondered whether subcortical gray matter structures were affected as well in autism. There has been some suggestion that medio-temporal lobe structures may be enlarged relative to overall brain enlargement in autism. Therefore, in **chapter 5**, we set out to investigate amygdala and hippocampus volumes in the same subjects, already described in chapter 3 and 4. Although a significant increase in absolute hippocampal volume was found, autistic subjects and controls showed no significant difference in amygdala and hippocampus volume once brain size was controlled for. Indeed, it was suggested previously that, if there were reliable differences in the morphology of medial temporal

lobe structures distinctive of autism, these would have undoubtedly been consistently reported by now [Bigler et al. 2003]. Accordingly, it was recently suggested that medial-temporal lobe pathology, specifically in the amygdala, might not be a characteristic of autism proper, but rather, might contribute to comorbid anxiety in autism [Amaral et al. 2003]. The absence of volumetric abnormalities in the medial-temporal lobe structures, however, does not exclude the existence of functional impairments sustaining a possible role in the pathophysiology of autism [Kwon et al. 2004]. To recap, we identified important morphological alterations in autism, although it is obvious that future (neuropathological) research is necessary to clarify the underlying mechanisms of global increase in gray matter volume and disproportional enlargement of the ventricles as found in autistic subjects.
As it has been estimated that over 90% of the etiology of autism is derived from genetic factors [Bailey et al. 1995], we set out to investigate to what extent brain enlargement is under genetic control. In **chapter 6**, brain volumes of biological non-affected parents of 'our' autistic subjects were compared to those of healthy, married, and matched control couples. Identifying structural brain abnormalities under genetic control is of particular importance because these could represent endophenotypes of autism [Skuse 2001]. Previously, biological parents of autistic subjects were shown to share specific behavioral [Piven et al. 1997;Piven and Palmer 1997;Wolff et al. 1988], cognitive [Baron-Cohen and Hammer 1997;Happé et al. 2001;Hughes et al. 1997], and biological [Fatemi et al. 2002a;Leboyer et al. 1999] characteristics with their autistic offspring. However, none of the brain volumes, previously found to be enlarged in autistic subjects, differed significantly between parents with an autistic proband and healthy control couples. It was suggested that the pathogenesis of autism likely involves multiple genetic and environmental effects [Bailey et al. 1995], with possible inheritance of risk factors from both parents [Cook, Jr. et al. 1997;Hallmayer et al. 1996]. Thus, increased brain volume in autism might be caused by the interaction of paternal and maternal genes, possibly with an additional effect of environmental factors. For example, in studies on schizophrenia, another highly heritable (estimated at 80%) psychiatric disorder [Cannon et al. 1998], it has recently been shown that decrease in white matter volume seemed to reflect the increased genetic risk to develop schizophrenia, whereas the decrease in gray matter volume was related to environmental risk factors [Hulshoff Pol et al. 2004]. This might explain the absence of increases in gray matter volume in parents of autistic probands.

The second part of this thesis (chapter 7 and 8) focused on the possible underlying neuropathological abnormalities in autism. First we examined the available neuropathological literature on autism (**chapter 7**). In short, classic neuropathological studies were quite consistent in reports of increased cell

packing density and smaller neuronal size in the limbic system. In addition, a decreased number of Purkinje cells in the cerebellum was repeatedly found. Furthermore, age-related morphological alterations were reported in the cerebellar nuclei and the inferior olive. Finally, features of cortical dysgenesis in the cerebral cortex, such as increased neuronal density, increased cortical thickness, ectopic gray matter, and poor differentiation of the gray-white boundary were found, suggestive of abnormalities in cortical lamination and apoptosis [Rorke 1994]. However, the majority of neuropathology data remain equivocal, which could have been due to lack of statistical power, resulting from small sample sizes and from the heterogeneity of the disorder itself, and to the inability to control for potential confounding variables such as gender, mental retardation, epilepsy, and medication status. Most importantly, however, was the lack of rigorous quantification techniques, such as design-based stereology. Therefore, for the first time, using high-precision design-based stereology, we set out to investigate cortical neuron number and density as well as volume of cortical gray matter, subcortical gray matter, and cerebral white matter in autistic patients and age-matched controls (**chapter 8**). Although autistic patients showed almost no differences in mean volumes of cortical gray matter, subcortical gray matter, white matter and whole hemisphere compared to controls, they had significantly higher mean total neuron numbers in the cerebral cortex than the controls. In addition, autistic patients had higher mean overall cortical neuron densities than the controls, which however did reach only trend level due to interindividual variability. As the increase was mainly due to the difference within the youngest pair, the findings pointed to an early insult.

Overall, we have combined neuroimaging and neuropathology techniques to investigate the underlying structural brain abnormalities in autism. We showed a global increase in gray matter volume of approximately 5%, proportional to the increase in brain volume both in high-functioning autistic children and adolescents and young adults, but not in their non-affected biological parents. In addition, these autistic patients demonstrated excessively enlarged ventricles, an effect that was still present once brain size was controlled for. Furthermore, we showed a significant increase in mean total neuron numbers in the cerebral cortex of autistic patients.
Thus, structural brain abnormalities associated with autism seem to be widespread. In the present thesis, we confirmed earlier reports of increased brain volume. In addition, at least in medication-naive high-functioning autistic patients, this increase, still present in adolescence, seems to be due to a global increase in gray matter volume, with an excessive increase in ventricular volume. Autism, however, is a heterogeneous disorder, which makes the generalizability of these findings limited. Indeed, Szatmari and colleagues suggested

that the underlying (genetic) mechanisms in subgroups of autistic subjects might be different [Szatmari et al. 1998]. Consistently, the postmortem data, including largely autistic patients who are mentally retarded, do not point towards increased brain volume in autism, although cortical neuron number was increased. Thus, increased brain volume, continuing into adolescence, might be restricted to certain subgroups, whereas increased neuron number may be an important feature of other subgroups of autistic subjects. We present a hypothesis of disrupted migration and pruning that may be relevant to autism. The final outcome of these aberrant developmental processes, however, is suggested to differ between subgroups of autistic subjects. Finally, we will address how to investigate this hypothesis in the near future.

A theory of disrupted migration and pruning processes

An accumulating body of evidence suggests that autism is associated with increased head circumference [Courchesne et al. 2003;Lainhart et al. 1997] and brain volume [Brambilla et al. 2003;Cody et al. 2002]. However, some authors suggest that, at birth, head circumference of infants, later diagnosed with autism, is comparable to that of typically developing children [Courchesne et al. 2003;Lainhart et al. 1997], and that this enlargement is not present until after the first few months of life [Courchesne et al. 2003]. An initial incomplete pruning, resulting in abundant synapses and cells, was suggested to underlie these findings [Belmonte et al. 2004;Frith 2003;Howard et al. 2000]. However, Courchesne and colleagues speculated that, as these abundant synapses and cells were not meant to function properly, they would eventually die, resulting in normalization of or even decrease in brain volume in later childhood [Courchesne et al. 2004]. We add to this plausible explanation that in those subgroups of autistic subjects, in which brain enlargement is still be present in adolescence, this excessive compensatory cell death does not take place. Instead, these autistic subjects may somehow be able to recruit these ‘extra’ cells, resulting in increased dendritic growth and thus, increased brain volume, which is still present in adolescence. However, as these abundant neurons were not meant to function in an appropriate way [Kemper and Bauman 1993], this may result in deviant activation patterns [Belmonte and Yurgelun-Todd 2003;Hazlett et al. 2004;Pierce et al. 2001]. Recent reports of abnormal brain connectivity [Belmonte et al. 2004;Just et al. 2004;McAlonan et al. 2004] are in line with this theory, as are the results of the first DTI study, showing abnormal white matter tracts in autism [Barnea-Goraly et al. 2004].

Although this proposed pruning problem can explain the *postnatal*, quantitative changes that have been reported here, it cannot account for observations of cortical dysgenesis in autism, that have been suggested to originate in the *prenatal* period [Bailey et al. 1998]. Therefore, both pre- and postnatal cortical developmental processes should be investigated in order to clarify the underlying etiological mechanisms as applied to autism. Using molecular genetics, proteomics, and gene expression techniques, this can be established. Although still in its infancy in the field of autism, these techniques have come up with some promising results. Purcell and colleagues reported a decrease in the longest of three isoforms (180 kDa) of the neural cell adhesion molecule (NCAM) (a developmentally regulated protein involved in regulating axon fasciculation, neuron outgrowth, and synaptic plasticity) in the cerebella of autistic patients [Purcell et al. 2001]. Recently, an association was found between autism and several polymorphisms of the NCAM gene, located on chromosome 7q22 [Bonora et al. 2004]. Jamain and colleagues pursued the involvement of cell adhesion molecules in the etiology of autism [Jamain et al. 2003]. Mutation in the neuroligin genes NLGN3 and NLGN4, both located on the X-chromosome and important in synapse formation, were found in sib-pairs affected with autism. However, in two more recent studies, these mutations could not be replicated [Gauthier et al. 2004;Vincent et al. 2004]. Fatemi and colleagues found a significant reduction in reelin (a signaling protein that plays a pivotal role in neuronal migration and correct lamination during the embryonic period [Skaar et al. 2004]) in the cerebella of autistic patients [Fatemi et al. 2001a]. Interestingly, abnormal levels of reelin during adulthood have been associated with behavioral deficits, reduced synaptic plasticity, and cognitive distortions such as psychosis [Fatemi et al. 2001a]. Recently, independent research groups found significant associations between autism and several polymorphisms in the reelin gene, located on chromosome 7q [Persico et al. 2001;Skaar et al. 2004;Zhang et al. 2002], although not consistently [Devlin et al. 2004;Krebs et al. 2002;Li et al. 2004]. In addition, Bonora and colleagues reported several missense mutations the reelin gene [Bonora et al. 2003].

Fatemi and colleagues also investigated two key-regulators of programmed cell death (apoptosis), Bcl-2 and p53, in the frontal, parietal, and cerebellar cortex of autistic subjects [Araghi-Niknam and Fatemi 2003;Fatemi et al. 2001b;Fatemi et al. 2001a;Fatemi and Halt 2001]. A significant reduction of the Bcl-2 protein - inhibiting programmed cell death in the developing brain- was found, contrary to a significant increase of p35 –a pro-apoptotic factor.

Recently, Conciatori and colleagues reported for the first time a direct link between genes and enlarged heads in autism [Conciatori et al. 2004]. An association was found between a HOXA1 polymorphism (playing a major role in brainstem and cranial morphogenesis) and enlarged head circumference. Other

genes, plausible as participants in the production of increased brain volume include BDNF and VIP, have found to be elevated in blood samples of neonates later diagnosed with autism [Nelson et al. 2001].

Although the results from neuropathology and molecular genetics, proteomics, and gene expression studies in autistic subjects are promising, animal models of autism are essential in advancing understanding of autism's neuropathology. In animal models one can test the function of specific genetic and non-genetic factors, and consequently, their possible etiological role in autism. However, to date no animal model has been developed that adequately mimics both the core behavioral deficits and the neuropathological abnormalities of autism in one and the same animal. Thus, at best 'fractional' animal models have been developed to date that either copy particular symptoms or specific brain alterations of autism. However, the animal model developed by Patterson and colleagues comes close to mimicking autism in all its aspects [Patterson 2002].

Mice lacking Dvl1 (a gene important in determining cell polarity) show abnormal gating and reduced social interaction. Mice lacking Oxt (the oxytocin gene) fail to change behavior on re-exposure to a known cage mate, thus reflecting an apparent lack of social memory. Male avpr1a (an arginine vasopressin receptor gene) knockout mice showed reduced anxiety-like behavior and a profound impairment in social recognition [Veenstra-VanderWeele et al. 2004]. Mouse mutants of Engrailed2 (EN2), a cerebellar patterning gene, displayed similar cerebellar morphological abnormalities, including a decrease in the number of Purkinje cells, as has been reported for autistic subjects [Gharani et al. 2004]. Interestingly, the human EN2 maps to 7q36, a chromosomal region that has demonstrated suggestive linkage to autism spectrum disorder [International Molecular Genetic Study of Autism Consortium (IMGSAC) 2001]. Falconer reported of the reeler mouse, with abnormalities in cerebellar development and a malformation of the cortex [Falconer 1951], long before comparable deviations were reported as a result of reduction in reelin in the cerebellar cortex of autistic subjects [Fatemi et al. 2001a]. Neonatal rats infected with Borna disease virus show neurobehavioral disturbances in sensorimotor, emotional, and social activity, together with a decrease in cerebellar Purkinje cells [Hornig et al. 2002]. Finally, the best animal model of autism so far showed atrophy of pyramidal cells despite normal cell proliferation rate and final enlargement of brain in offspring of mice exposed to human influenza virus during pregnancy [Fatemi et al. 2002b]. In addition, these mice displayed deficits in prepulse inhibition in the acoustic startle response, in exploratory behavior, and in social interaction [Shi et al. 2003].

Possible future directions

As has been shown, many factors can contribute to deviant migration and pruning processes and many alterations in brain development can result in autistic-like behavior. Animal models based on genetic, neurochemical, and behavioral manipulations offer the possibility of exploring the basic processes of brain development in detail, whereas human (neuropathology) studies can focus on defining subgroups of autism.

Thus, in the near future, the focus of research should be two-fold: first, effort should be put into establishing valid animal models of autism. Approaches to mimic autism in animal models should involve modeling specific neuropathological findings (such as increased neuron number) as well as particular behaviors (such as abnormal social interactions), ultimately in one and the same animal. Second, the implementation of stereology in human neuropathological research in separate homogeneous groups of autistic subjects holds great promise and might eventually result in the elucidation of the etiologies of autism.

Thus, although identification of predisposing factors of autism is hampered by the large degree of convergence from causal factors to altered brain development, and divergence from abnormal brain development into altered cognition and behavior, combinations of animal research, rigorous quantitative histologic techniques and molecular analyses will bring us closer to unraveling the underlying etiological factors of autism.

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Nederlandse samenvatting

Nederlandse Samenvatting

Autisme is een ontwikkelingsstoornis die enerzijds gekenmerkt wordt door kwalitatieve tekortkomingen in de sociale interactie en de communicatie en anderzijds door beperkte en rigide gedragspatronen. Symptomen zijn meestal zichtbaar ruim voordat het kind 3 jaar oud is en blijven het hele leven aanwezig, al worden ze over het algemeen minder ernstig met het ouder worden. Ongeveer 1 à 2 op de 1000 kinderen lijden aan autisme, waarbij jongens vier keer zo vaak zijn aangedaan als meisjes. Ondanks dat vroeger gedacht werd dat autisme veroorzaakt werd door perfectionistische en kille ouders, is door het onderzoek van de afgelopen decennia, onomstotelijk vast komen te staan dat autisme een aandoening van de hersenen is. Het is echter nog onvoldoende duidelijk wanneer het misgaat in de hersenen van mensen met autisme en wat er dan precies misgaat. Wel is ondertussen duidelijk geworden dat genetische factoren een zeer belangrijke rol spelen in het ontstaan van autisme.

Dit proefschrift is geschreven om enerzijds de kennis die we tot nu toe hebben over hersenafwijkingen in autisme te bundelen en anderzijds om deze kennis te vergroten door zelf een aantal onderzoeken uit te voeren. Het uiteindelijke doel was om een stukje dichter bij het ontrafelen van het raadsel "autisme" te komen.

Allereerst hebben we 2 reviews geschreven over alle tot nu toe gepubliceerde studies waarin onderzoek gedaan is naar structurele hersenafwijkingen in autisme, ofwel met behulp van structurele magnetische resonantie imaging (MRI), een beeldvormende techniek, ofwel door middel van postmortem onderzoek. Ten tweede hebben we zelf 4 structurele MRI studies uitgevoerd, 3 waarin we hersenvolumes van hoogfunctionerende medicatienaïeve kinderen en adolescenten met autisme hebben onderzocht en 1 waarin we hersenvolumes van hun ouders onderzocht hebben. Ten derde hebben we met behulp van postmortem onderzoek gekeken naar wat de cellulaire basis zou kunnen zijn van de hersenafwijkingen zoals die gevonden zijn met MRI. Tot slot hebben we een hypothese gevormd die alle bevindingen van dit proefschrift kan verklaren.

In **Hoofdstuk 2** hebben we een uitgebreide samenvatting gemaakt van de structurele hersenafwijkingen in autisme, zoals die gevonden zijn met MRI onderzoek. In het kort kan gezegd worden dat een toename in het hersenvolume de meest consistent gevonden afwijking is, alhoewel het nog onduidelijk is of deze hersenvergroting alleen op heel jonge leeftijd aanwezig is of dat ze tot in de adolescentie of zelfs de volwassenheid blijft bestaan. Tevens is er nog geen eenduidig antwoord op de vraag of deze hersenvergroting te wijten is aan een toename in het grijze stof volume (grofweg het gedeelte van de hersenen waar de zenuwcellen zich bevinden) of het witte stof volume (grofweg het gedeelte van de hersenen dat de verbindingsbanen tussen de afzonderlijke

zenuwcellen vormt). Tot slot is ook onduidelijk of de hersenvergroting een globaal verschijnsel is of meer gelokaliseerd is in specifieke hersendelen. Hoewel de resultaten betreffende het cerebellum vrij consistent een vergroting laten zien en het corpus callosum bijna altijd kleiner wordt gevonden, zijn de resultaten van andere structuren, zoals de amygdala, de hippocampus, en de afzonderlijke hersenkwabben veel minder eenduidig. Kortom, de bevindingen zijn vrij tegenstrijdig. Dit kan deels verklaard worden door het feit dat de onderzochte groepen te klein waren, zeker gezien het feit dat autisme een zeer heterogene stoornis is, deels ook door het niet corrigeren voor factoren die van invloed kunnen zijn op de uiteindelijke resultaten, zoals leeftijd, geslacht, IQ, sociaal-economische status en het al dan niet gebruiken van medicijnen.
Om een aantal van de bovengenoemde vragen te kunnen beantwoorden, hebben we de hersenanatomie van homogene groepen van hoogfunctionerende medicatienaïeve kinderen, adolescenten en jong volwassenen met autisme (patiënten) onderzocht en vergeleken met die van groepen gezonde vrijwilligers die qua leeftijd, geslacht, IQ, lengte, gewicht, handvoorkeur en opleiding vergelijkbaar waren met de patiënten. In **Hoofdstuk 3 en 4** hebben we laten zien dat zowel hoogfunctionerende medicatienaïeve kinderen als adolescenten en jong volwassenen met autisme een globale vergroting van zowel het cerebrum (de grote hersenen) als het cerebellum (de kleine hersenen) van ongeveer 5% vertonen. Deze vergroting werd veroorzaakt door een toename in het volume van de grijze stof en niet door een toename van het volume van de witte stof. Daarnaast hebben we een excessieve vergroting van de ventrikels (de hersenkamers) gevonden, een vergroting die op kinderleeftijd 45% bedroeg en in jong volwassenheid zelfs 70% bedroeg. Alhoewel eerdere onderzoekers gevonden hebben dat de toename in hersenvolume alleen aanwezig zou zijn bij hele jonge autistische kinderen (waarvan het overgrote deel ook mentaal geretardeerd was) veronderstellen wij dat in hoogfunctionerende individuen met autisme, deze hersenvergroting in ieder geval blijft bestaan tot in jong volwassenheid. Aangezien verscheidene studies een significante positieve correlatie tussen hersenvolume en IQ hebben gevonden en andere studies significant kleinere hoofdomtrekken in individuen met mentale retardatie hebben gevonden, zou het dus heel goed mogelijk zijn dat de toename in hersenvolume, veroorzaakt door autisme, tenietgedaan wordt door het gelijktijdig aanwezig zijn van mentale retardatie. Kortom, met de resultaten uit de 2 net beschreven studies kunnen drie vragen beantwoord worden. De hersenvergroting in autisme, tenminste in hoogfunctionerende individuen, lijkt aanwezig te blijven tot in jong volwassenheid, lijkt veroorzaakt te worden door een toename in grijze stof volume en lijkt globaal te zijn. Helaas kunnen we met MRI alleen speculeren over de mogelijke onderliggende mechanismen die tot deze vergroting hebben geleid. Zowel toegenomen neurogenese (aanmaak van neuronen oftewel zenuwcellen), als toegenomen gliogenese (aanmaak van

steuncellen) als afgenomen apoptose (geprogrammeerde celdood) en andere 'snoeiprocessen' zouden ten grondslag kunnen liggen aan een vergroting van het grijze stof volume. Opmerkelijk is dat één van de functies van serotonine, een neurotransmitter waarvan bekend is dat de concentratie ervan in het bloed van mensen met autisme sterk is toegenomen, het blokkeren van de apoptose is. Theoretisch zou deze zogenaamde hyperserotoninemie dus kunnen leiden tot een excessieve remming van de apoptose. De bevinding van de enorme groei in ventrikel volume, die ook nog eens lijkt toe te nemen met de leeftijd, was, hoewel enigszins onverwachts, interessant. Eén verklaring zou kunnen zijn dat structuren in de hersenen, die rondom de ventrikels liggen, verkleind zouden zijn. Echter, het ondersteunende bewijs hiervoor is zeer mager. Men zou ook kunnen redeneren dat kinderen met autisme die jonger dan 'onze' kinderen zijn (dus jonger dan 7 jaar) wellicht een meer dan 5% hersenvergroting vertonen, hetgeen impliceert dat, alhoewel individuen met autisme op jong volwassen leeftijd nog steeds grotere breinen hebben dan controle personen, ze toch met de leeftijd meer hersenweefsel verliezen. Waar hersenweefsel verdwijnt is het zeer waarschijnlijk dat er hersenvocht voor in de plaats komt, hetgeen dus zou resulteren in vergrote ventrikels. Het is duidelijk dat dit allemaal speculaties zijn, interessant, maar zeker niet bewezen.

Na de bevinding van een globale vergroting van de grijze stof van het cerebrum (de zogenaamde hersenschors of cortex), waren we benieuwd of kleine grijze stof kernen diep in de hersenen ook vergroot zouden zijn. Met name de structuren in de mediale temporaal kwab, zoals de amygdala en de hippocampus, zijn interessant, gezien hun functie in sociaal gedrag, geheugen en stimulus integratie. In **Hoofdstuk 5** presenteren we dan ook een MRI studie waarin we de volumes van de amygdala en de hippocampus hebben gemeten in dezelfde subjecten als beschreven in Hoofdstuk 3 en 4. Het volume van de amygdala verschilt niet tussen patiënten en controles. Het volume van de hippocampus is weliswaar significant groter in de patiënten groep, maar deze vergroting is evenredig aan de al eerder gerapporteerde vergroting van de gehele hersenen. Kortom, wij hebben geen bewijs gevonden voor een specifieke vergroting van de structuren in de mediale temporaal kwab in autisme, hetgeen overeen lijkt te komen met de recente veronderstelling dat de amygdala niet zozeer van belang is bij sociaal gedrag. Daarnaast sluit deze negatieve bevinding natuurlijk geenszins uit dat er eventueel wel functionele afwijkingen in de amygdala en de hippocampus aanwezig kunnen zijn. Samenvattend kan gezegd worden dat we belangrijke morfologische veranderingen hebben gevonden in autisme. Vanzelfsprekend echter, is toekomstig (postmortem) onderzoek onontbeerlijk om de onderliggende oorzaken van een toegenomen grijze stof volume op te helderen.

Zoals heel kort aangestipt, spelen genen een belangrijke rol in het ontstaan van autisme. Volgens schattingen zou autisme voor ongeveer 90% genetisch bepaald zijn, hetgeen voor ons de aanleiding vormde om te onderzoeken in welke mate de hersenvergroting van 'onze' patiënten ook onder genetische controle stond. Om dit te onderzoeken hebben we in **Hoofdstuk 6** de hersenvolumes van niet aangedane ouders van 'onze' patiënten populatie vergeleken met die van controle echtparen. Eerdere studies hadden reeds aangetoond dat ouders met een autistisch kind verscheidene gedragingen (zoals bijvoorbeeld een gereserveerde houding ten opzichte van andere mensen) delen met hun kind. Tevens komt een aantal cognitieve kenmerken, passend bij autisme (zoals bijvoorbeeld moeite met het zich in andere mensen te verplaatsen en moeite met het overzien van het grote geheel), vaker voor bij ouders met een autistisch kind dan ouders zonder een autistisch kind. Hetzelfde geldt voor bepaalde biologische maten, zoals bijvoorbeeld een verhoogd serotonine gehalte in het bloed. Over de eventuele erfelijkheid van een vergroot hersenvolume in autisme was nog niks bekend. Wij hebben aangetoond dat de hersenvolumes van ouders met een autistisch kind op geen enkele manier significant verschillen ten opzichte van die van controle echtparen. Vaders noch moeders vertoonden verschillen in hersenvolumes wanner ze vergeleken werden met de controle mannen respectievelijk vrouwen. De etiologie van autisme is hoogstwaarschijnlijk afhankelijk van meerdere genen, mogelijk afkomstig van zowel vader als moeder. De meest voor de hand liggend verklaring voor de afwezigheid van hersenvergroting in de ouders is dan ook dat deze hersenvergroting veroorzaakt wordt door zowel genen van vader als van moeder, waarschijnlijk met een additioneel effect van omgevingsfactoren. Interessant om in dit kader te vermelden is dat bij schizofrenie, een andere sterk genetisch bepaalde psychiatrische aandoening, inderdaad dit tweeledige effect gevonden wordt. De afwijkingen in de witte stof lijken een genetische grondslag te hebben, terwijl de afwijkingen in de grijze stof voornamelijk door omgevingsfactoren bepaald lijken. Dit zou kunnen verklaren waarom wij de afwijkingen die wij in de grijze stof vinden in autisme, niet terug kunnen vinden in de gezonde ouders.

Het tweede deel van het proefschrift richt zich op de mogelijke onderliggende neuropathologische afwijkingen in autisme. Als eerste hebben we, in navolging van het MRI gedeelte, in **Hoofdstuk 7** in kaart gebracht wat er al bekend was over de neuropathologie van autisme. Samengevat laten klassieke neuropathologische studies zien dat ongeveer een kwart van de individuen met autisme een vergroot hersengewicht heeft. Verder zijn de neuronen in het limbische systeem kleiner en zitten ze dichter op elkaar gepakt. Daarnaast is consistent een afname in het aantal Purkinje cellen in het cerebellum gerapporteerd. Verder zijn er leeftijdsgebonden afwijkingen in cerebellaire kernen en in de

olijfkern gevonden. Ten slotte is een aantal kernmerken van corticale dysgenese gevonden, zoals toegenomen celdichtheid, toegenomen dikte van de cortex, het aanwezig zijn van neuronen op plaatsen waar ze eigenlijk niet horen en een onduidelijke scheiding tussen de grijze en de witte stof, stuk voor stuk verschijnselen die op een stoornis in de corticale laminatie en het apoptose proces wijzen. Echter, de meerderheid van de neuropathologie bevindingen zijn niet eenduidig, wat deels te wijten is aan de kleine studies (per studie vaak slechts één of enkele patiënten), de heterogeniteit van autisme en het niet controleren voor factoren zoals geslacht, mentale retardatie en epilepsie. Maar wat een nog belangrijkere reden lijkt te zijn is het gebrek aan goed opgezette kwantitatieve studies, zoals stereologische studies. Daarom zijn wij, voor de eerste keer ter wereld in het autisme veld, gestart met een stereologie studie. In **Hoofdstuk 8** beschrijven we hoe we postmortem (n=6) het volume van de corticale grijze stof, de subcorticale grijze stof en de witte stof hebben bepaald. Daarnaast hebben we het aantal en de dichtheid van de neuronen in hemisferen van kinderen en adolescenten met autisme en in controle hemisferen bepaald. We hebben aangetoond dat, hoewel er qua volume geen verschil was tussen de hemisferen van patiënten en controles, er significant meer neuronen aanwezig waren in de cortex van patiënten. Tevens was de dichtheid van de neuronen hoger in de patiënten groep, al bereikte dit verschil ten opzichte van de controles net geen significantie. Aangezien het verschil in aantal neuronen tussen patiënten en controles het allergrootst was op jonge leeftijd, lijkt er sprake te zijn van een vroege beschadiging.

In dit proefschrift hebben we neuroimaging en neuropathologie technieken gecombineerd om een beter beeld te kunnen krijgen van de structurele hersenafwijkingen zoals die zich voordoen in autisme. We hebben aangetoond dat hoogfunctionerende medicatienaïeve kinderen, adolescenten en jong volwassenen met autisme, maar niet hun ouders, een globale vergroting van de grijze stof van ongeveer 5% vertonen, een vergroting die evenredig is aan de vergroting van het totale hersenvolume. Daarnaast vertonen de patiënten met autisme een excessieve ventrikelvergroting, een vergroting die ook nog eens toe lijkt te nemen met de leeftijd. Ten slotte hebben we aangetoond dat de hersenen van individuen met autisme significant meer corticale neuronen bevatten.

Structurele hersenafwijkingen in autisme lijken dus wijdverspreid te zijn. In dit proefschrift hebben we eerdere bevindingen van een vergroot hersenvolume bevestigd en toegevoegd dat de vergroting, in ieder geval in hoogfunctionerende medicatienaïeve individuen met autisme, nog steeds aanwezig is in jong volwassenheid en veroorzaakt lijkt te worden door een globale toename in grijze en niet in witte stof volume. Echter, omdat autisme een heterogene

stoornis is, zijn de bevindingen niet zo maar te generaliseren naar alle individuen met autisme. Er is inderdaad ook door andere onderzoekers gesuggereerd dat de onderliggende mechanismen van autisme in subgroepen verschillend zouden kunnen zijn. Het feit dat wij in onze postmortem studie, waarin vooral patiënten met mentale retardatie geïncludeerd waren, geen vergroot hersenvolume hebben gevonden is hiermee in overeenstemming. Het lijkt er dus op dat een vergroot hersenvolume, dat tot in volwassenheid aanwezig blijft, slechts in specifieke subgroepen van patiënten voorkomt, terwijl een toename van het aantal neuronen voorbehouden is aan andere subgroepen. Wij hebben in **Hoofdstuk 9** een hypothese geformuleerd die de bevindingen uit de MRI en de neuropatholgie studies in dit proefschrift integreert. Samengevat stelt deze hypothese dat een stoornis in de migratie van neuronen en het zogenaamde snoeiproces (pruning) ten grondslag zou kunnen liggen aan autisme. Wanneer neuronen die normaal gesproken zouden moeten afsterven omdat ze niet (meer) nodig zijn, blijven leven ontstaat er een overschot aan neuronen (en als consequentie, een vergroot volume). Echter, daar deze 'extra' neuronen niet voorbestemd waren om lang te overleven zal in een aantal gevallen dit overschot aan neuronen uiteindelijk toch sterven hetgeen zou leiden tot een normalisatie van het aantal neuronen (en dus normalisatie van het hersenvolume). Als er echter een manier gevonden kan worden om deze overvloedige neuronen toch te gebruiken blijven ze bestaan en blijft dus ook het vergrote hersenvolume bestaan. Echter, omdat deze neuronen niet voorbestemd waren tot een langdurige functie zullen ze mogelijk leiden tot een afwijkend activatie patroon van de hersenen, hetgeen inderdaad recent meerdere malen gevonden is in autisme. De verbindingen tussen neuronen onderling zullen ook afwijkend verlopen, een verschijnsel dat ook al vaker gerapporteerd is.
Afsluitend kan gesteld worden dat er door MRI en postmortem onderzoek veel bekend is geworden over afwijkende hersenstructuren in autisme, maar dat er ook nog erg veel onbekend is. Met toekomstig kwantitatief neuropathologisch onderzoek kan de cellulaire basis beter in kaart gebracht worden. Daarnaast denken we dat ook de genetica een grote rol zal spelen in het verder ontrafelen van de onderliggende oorzaak van autisme.

Curriculum Vitae

Curriculum Vitae

Saskia Palmen werd geboren op 2 januari 1974 te Delft. In 1992 behaalde ze haar gymnasium β diploma aan de Stedelijke Scholengemeenschap in Maastricht. In 1999 rondde ze cum laude haar geneeskunde studie aan de Vrije Universiteit te Amsterdam af, waarna ze een maand later startte als promovendus bij het Rudolf Magnus Instituut voor Neurowetenschappen, Afdeling Kinder- en Jeugdpsychiatrie van het Universitair Medisch Centrum Utrecht. In 2004 is zij voor 6 maanden vertrokken naar de afdeling Anatomie en Cel Biologie van de Universiteit Aken, RWTH te Duitsland om daar het neuropathologische deel van haar promotieonderzoek uit te voeren. Na de verdediging van dit proefschrift op 22 maart 2005 zal zij 1 april starten met de opleiding tot kinder- en jeugdpsychiater in het UMC Utrecht.

Saskia Palmen was born on January 2nd 1974 in Delft, the Netherlands. In 1992 she graduated from the Stedelijke Scholengemeenschap Maastricht. In 1999, she completed cum laude her medical degree at the Free University, Amsterdam. One month later she started her PhD project at the Rudolf Magnus Institute of Neuroscience, Department of Child and Adolescent Psychiatry at the University Medical Center Utrecht. In 2004, she spent 6 months at the Department of Anatomy and Cell Biology, RWTH Aachen University, Aachen, Germany to perform the neuropathological part of her thesis. After defending her thesis on March 22nd 2005, she will start her training to become a childpsychiatrist at the University Medical Center Utrecht.

List of Publications

List of Publications

Journal articles

Palmen SJMC, Heinsen H, Van Engeland H, Steinbusch HWM, Von Cappeln P, Korr H, Hof PR, Schmitz C. Cortical neurons are more numerous in autism. Submitted

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Dankwoord

Dankwoord

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