Toddlers with autism
metabolic, radiologic and volumetric aspects of brain development

Mijke Zeegers

The studies described in this thesis were supported by grants 940-38-045, 940-38-014 (Chronic Disease Program), and by grant 28.3000-2 of the Preventiefonds-ZONMW, by the Netherlands Organisation for Scientific Research (NWO), by a grant from the Dutch Ministry of Health, Welfare and Culture, and by grants from Cure Autism Now, the Korczak Foundation and the Dutch Brain Foundation. They were performed at the Department of Child and Adolescent Psychiatry, University Medical Center Utrecht, the Netherlands.

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Thesis Utrecht University

ISBN-10: 90-393-4343-8
ISBN-13: 978-90-393-4343-2

Design and lay-out: Dorine Verharen bNO
Printed by: Optima Grafische Communicatie

Toddlers with autism:
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Peuters met autisme:
metabolische, radiologische en volumetrische
aspecten van hersenontwikkeling
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. W.H. Gispen, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op

vrijdag 17 november 2006 des ochtends te 10.30 uur door

Mijke Zeegers

geboren op 14 september 1976 te Helmond, Nederland

Promotoren	Prof. Dr. J.K. Buitelaar
	Prof. Dr. H. van Engeland
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	Dr. H.E. Hulshoff Pol

Financial support for the publication of this thesis by the Prof. Dr. L.N.J. Kamp Stichting is gratefully acknowledged.

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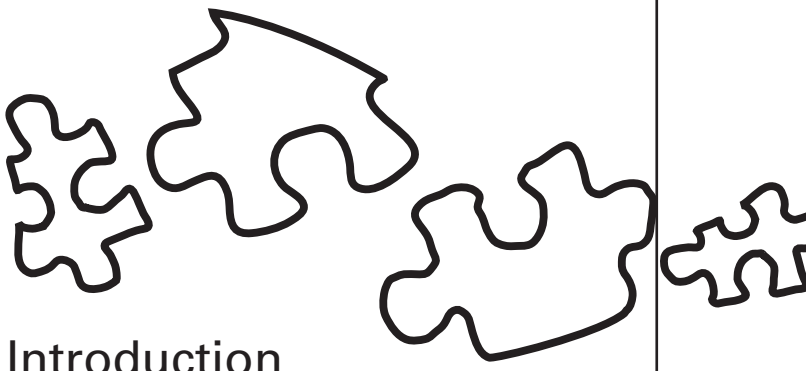
"The best thing for being sad," replied Merlin, "is to learn something. That is the only thing that never fails. You may grow old and trembling in your anatomies, you may lie awake at night listening to the disorder of your veins, you may miss your only love, you may see the world about you devastated by evil lunatics, or know your honour trampled in the sewers of baser minds. There is only one thing for it then – to learn. Learn why the world wags and what wags it. That is the only thing which the mind can never exhaust, never alienate, never be tortured by, never fear or distrust, and never dream of regretting. Learning is the thing for you. Look at what a lot of things there are to learn."

T.H. White "THE ONCE AND FUTURE KING"

Voor Ruud,
mijn vader
Cleo's opa



Introduction

The spectrum of autistic disorders (American Psychiatric Association, 1994) includes autistic disorder or autism, Asperger's syndrome and Pervasive Developmental Disorders, Not-Otherwise-Specified (PDD-NOS). Spectrum disorders are diagnosed based on problems in the areas of social development, communication, and play, and restricted and stereotyped interests (with social factors being given slightly more weight). All disorders within the spectrum are characterised by what is known as "The Triad": 1) qualitative impairments in social interaction as shown by the use of non-verbal behaviours, a lack of spontaneous showing and sharing of interests and a lack of social emotional reciprocity; 2) qualitative impairments in social communication shown by a delay in language development without non-verbal compensation, problems initiating and sustaining conversations, repetitive and stereotyped use of language and a lack of varied and imaginative or imitative play; and 3) a restricted repertoire of interests, behaviours and activities as shown by an abnormal focus on particular topics, an adherence to non-functional routines or rituals, repetitive stereotyped motor mannerisms and a preoccupation with parts of - rather than whole - objects. All of these characteristics can be present in varying degrees of severity. See also table 1 (page 12).

It is not possible to derive a precise figure for the current true incidence of autism spectrum disorder (ASD), because of uncertainty over the boundaries of the syndrome. However, in 2001, autism prevalence was estimated to be 16.8 per 10.000. The prevalence of ASD other than autism was 45.8. The total rate of ASD provided by the sum of these two figures is 62.6 per 10.000 (Chakrabarti and Fombonne, 2001). Other studies report prevalence estimates of 10 cases per 10.000 for autistic disorder and 2.5/10.000 children for Asperger's syndrome (Fombonne, 2003). Therefore, if the rate for ASD is approximately 30 to 60 cases per 10.000, with about a quarter of those meeting full criteria for autism, this is a huge increase over the

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

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

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

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

Table 1 *Diagnostic criteria according to DSM-IV*

original estimate 40 years ago of 4 per 10.000 (Lotter, 1966). Several factors complicate the interpretation of this apparent increase, including changes in diagnostic practice, increased awareness of the disorder, earlier diagnosis, issues of study design and case ascertainment, and the problem of 'diagnostic substitution' (e.g., choosing to use a label of autism as opposed to a label of mental retardation for educational or subsidiary purposes) (Croen et al., 2002; Wing and Potter, 2002; Fombonne, 2001). Most researchers consider the increase largely a consequence of improved ascertainment and a considerable broadening of the diagnostic concept. However, a true risk due to some, yet to be identified, environmental risk factor cannot be ruled out. There is no support for a role of either the MMR vaccine or thimerosal in causation, but the evidence on the latter is more limited (Rutter, 2005).

As prevalence studies have yielded higher estimates of affected individuals, the IQ distribution of ASD has also shifted. In earlier studies, two-thirds or more of children with autism were reported to be mentally retarded. Current epidemiological studies estimate that fewer than half of the children with more broadly defined ASD have nonverbal IQs under 70 (Chakrabarti and Fombonne, 2001). There is a higher incidence of autism in boys than in girls, with reported ratios averaging around 3.5 or 4.0 to 1 (Fombonne, 2003; Lord et al., 1982). There is a strong association between mental handicap and sex ratio; the highest male:female ratios are reported in individuals functioning in the normal IQ range and the lowest male:female ratios are found in individuals with autism and profound mental retardation (Lord et al., 1982).

In the Chakrabarti and Fombonne study, of the children with ASD, 9.3% had an associated medical condition that was likely to have played a part in causation (Chakrabarti and Fombonne, 2001). Associations with autism have been noted for numerous medical conditions (Gillberg and Coleman, 2000). The strongest association with a medical condition is that of autism with epilepsy, with two peaks of onset in both early childhood and adolescence (Rutter, 1970). The mean rate of epilepsy was estimated as 16.8%, although this is likely an underestimate given the median age of available samples and the increased risk for onset of seizures throughout childhood, adolescence and early adult life (Gillberg and Coleman,

2000; Rutter, 1970). Fragile X syndrome and tuberous sclerosis have also often been associated with autism (Smalley, 1998; Rutter et al., 1994; Hagerman et al., 1992).

There is ample evidence to suggest that autism is largely genetically determined. Twin studies show a 60-90% concordance rate in monozygotic twins, contrary to the 0-20% concordance in dizygotic twins (Bailey et al., 1995; Steffenburg et al., 1989; Ritvo et al., 1985). The recurrence ratio in siblings is estimated to range from 50 to 150 (Jorde et al., 1991; Folstein and Piven, 1991; Smalley et al., 1988). Together, epidemiological, twin and family data suggest that the vast majority of cases of ASD arise on the basis of a complex genetic predisposition, perhaps involving interactions between as few as three or four susceptibility loci, but more likely are estimations of 10 loci or more (Risch et al., 1999; Pickles et al., 1995).

Behaviour

Early signs of autism include a lack of social interest in the first months of life, - with reduced levels of social engagement and social-communicative intent, whereas differences in non-social areas are much less striking. By age 6 to 12 months, differences become more pronounced in the communicative area, including a general lack of orientation toward verbalisation in general and to their own name in particular. Infants with autism are less interested in people at a time when most infants begin to more fully integrate object exploration with social interaction and become more clearly intentional (Bates et al., 1979).
Preschool children with autism fail to assume anticipatory postures, reach for familiar persons, show interest in children other than peers, and engage in simple social interaction games - all are social behaviours that normally would be expected prior to 12 months (Klin et al., 1992). Items that differentiate children later diagnosed as autistic from typically developing children include social activities (seeking shared enjoyment, social reciprocity, use of another person as a tool, interest in other children), and communicative tasks (attending to voice, pointing, using and understanding gesture) (Lord, 1995). As overall social engagement and responsivity is limited, so is eye contact (Dawson et al., 2000). Play is less purposeful, less symbolic, and less developmentally complex (Sigman et al., 1999; McDonough et

al., 1997; Stone et al., 1990; Mundy et al., 1986). Difficulties in the area of joint attention are striking (Sigman et al., 1999; Mundy et al., 1990) and such behaviours are central in development of communicative and social-cognitive abilities (Tomasello, 1995).

Diagnosis at an early age

For children school age and up, the clinical diagnosis of autism is highly stable, with 72% to 87% of cases retaining the diagnosis at follow-up (Klin et al., 2004). Diagnosing younger children remains more challenging. There is now substantial evidence that autism can be reliably diagnosed in children as young as two years (Moore and Goodson, 2003; Lord, 1995), though there is more variability in diagnosis at follow-up in children with early diagnoses of atypical autism or PDD-NOS (Charman and Baird, 2002; Cox et al., 1999; Stone et al., 1999). Probably because of the absence of early language delay, it seems that Asperger syndrome tends to be diagnosed rather later than autism (Gilchrist et al., 2001). To complicate the task even further, early symptoms change over time (Lord, 1995; Kanner, 1968). There seem to be significant changes between the second and third birthday, with higher levels of more "typical" autistic behaviours present by age three (Lord and Pickles, 1996; Lord, 1995).

Diagnosis at an early age is difficult. Some of the DSM-IV criteria are clearly not suitable for very young children, e.g., criteria involving peer relationships and conversational skills. The Autism Diagnostic Interview – Revised (ADI-R, Lord et al., 1994) works well for children over 4 years of age but much less so for younger children (Cox et al., 1999; Stone et al., 1999; Lord, 1995). Some children at age two exhibit the social and communication-play problems typical of autism, but do not yet exhibit the unusual stereotyped movements or other behaviours in the third category of disturbance - though many of these children go on to do so before their third birthday (Volkmar et al., 2005). Furthermore, there are continuing uncertainties about how to combine the ADI-R and Autism Diagnostic Observation Schedule (ADOS, Lord et al., 1989) assessments and what to do with the diagnosis when the two measures give different answers (Chakrabarti and Fombonne, 2001). To date, the most robust method to diagnose autism in infants and very young children

remains by the experience of clinicians familiar with this group (Cox et al., 1999; Stone et al., 1999; Lord, 1995).

Regression

The most commonly reported onset of autistic disorders is a gradual course in which certain behaviours or lack of behaviours cause parental concern during the child's first two years, resulting in a diagnosis being given sometime between three and four years of age. Often, parents report that although they did not begin to notice symptoms until at least the second year of life, with hindsight they think that behavioural abnormalities were present much earlier, even in some cases during the first few months (Lord, 1995).

The second pattern of early course is characterised by normal or near normal development followed by a loss of skills or regression during the first or second year of life (Rogers, 2004; Tuchman and Rapin, 1997). Estimates of the prevalence of this late onset pattern range from 20 to 49% (Davidovitch et al., 2000; Kurita, 1996; Hoshino et al., 1987). In some children, there may be a gradual or more rapid loss of language and/or social skills (Tuchman and Rapin, 1997; Rogers and DiLalla, 1990). In other cases, the problem seems to be not so much of loss as of a failure to make progress, e.g., the child seems to say one or two words, but then language does not progress (Siperstein and Volkmar, 2004). After the regression, children generally do not begin to regain skills immediately, and very few or none recover fully (Rapin and Katzman, 1998). In general, apparent early onset of autism has not been associated with greater severity of symptoms (Rogers and DiLalla, 1990).

Theories of autism

There are several theories addressing the symptoms and clinical course associated with autism. The most influential framework that emerged in the 1990's was the Theory of Mind hypothesis. It suggests that the social dysfunction in autism is the result of disruptions in processes of learning how to conceive other people's and one's own mind (Baron-Cohen, 1995). Individuals with autism may have difficulty in making attributions of mental states to others and to themselves. This makes it

difficult for them to construct a social world, guided by intentions, desires, and beliefs (Baron-Cohen et al., 2000).

Drawing upon assumptions from gestalt psychology (e.g. (Koffka, 1935)), the Weak Central Coherence hypothesis of autism (Happe and Frith, 1996; Frith, 1989) focuses on the tendency to process all stimuli in a fragmented fashion (a piecemeal processing style), focusing on details (localised processing) rather than integrated and meaningful wholes (configural processing) (Frith, 1989). It tries to explain the remarkable skills (the "islets of ability" that often appear in the midst of otherwise limited intelligence) as well as the profound deficits of autism in terms of a single underlying cognitive dysfunction or processing style (Happe, 1999; Shah and Frith, 1993). The restricted and repetitive interests and behaviours that constitute the third leg of the diagnosis are argued to be manifestations of a weak "drive" for central coherence. Yet, as Happé has pointed out, the Weak Central Coherence account of autism remains "loosely defined and conceptualised" (Happe, 1997).

The Executive Dysfunction hypothesis attempts to integrate the inflexible and rigid behaviour of autistic individuals with the impaired ability to engage in reciprocal social-communicative interactions. Executive function has been conceptualised as involving several overlapping but potentially dissociable mental operations, such as planning, working memory, maintenance and shifting of mental set, and inhibition of prepotent responses. Successful social functioning, much like the card-sorting test, requires "integration and weighing of multiple contextual variables, selective attention to relevant aspects of the environment, and inductive logic" (Rumsey, 1985). Individuals with autism are hypothesised to have difficulties with such mental operations.

The Extreme Male Brain Theory proposes that individuals on the autistic spectrum are characterised by impairments in empathising alongside intact or even superior systemising. Empathising involves recognising what another person may be feeling or thinking, and responding to those feelings with an appropriate emotion of one's own. Systemising, on the other hand, involves identifying the laws that govern how a system works. Once the laws are known, one can control the system or predict its behaviour. Both are considered key dimensions in defining the male and female brain. Reduced empathy in people with ASD is evident in their lower scores on emotion recognition tests (Baron-Cohen et al., 2001), the empathy quotient (Baron-

Cohen and Wheelwright, 2004), the friendship and relationship quotient (Baron-Cohen and Wheelwright, 2003), and tests of social sensitivity such as the ''faux pas'' test (Baron-Cohen et al., 1999). Intact or even superior systemising is suggested by their higher scores on the systemising quotient (Baron-Cohen et al., 2003), and the embedded figures test (Jolliffe and Baron-Cohen, 1997) - although it is unclear if the latter is really a test of systemising, or simply a test of good attention to detail. It is also suggested by their strong obsessions, or areas of narrow interest, which tend to focus on systems (Baron-Cohen and Wheelwright, 1999). In low functioning children, characteristic behaviours such as 'insistence on sameness', repetitive behaviour, obsessions with lawful systems (e.g., train timetables), islets of ability (e.g., calendrical calculation), precocious understanding of machines, and superior attention to the detection of change all involve a strong interest in rule-based prediction and therefore can be interpreted as signs of hypersystemising.

A fifth hypothesis, the Limbic System hypothesis, is less a coherent psychological theory, than a collection of biological, neuropsychological, and behavioural findings that have connected this network of the brain to autism. It proposes that impairment of psychological functions, traditionally associated with the medial temporal lobes and limbic brain structures, may best explain the profound social and communicative deficits that characterize autism (Joseph, 1999).

The Theory of Mind, Weak Central Coherence theory, and Executive Dysfunction framework have been the most active foci for psychological studies in the past decade - because of clinical face validity and research productivity (Happe and Frith, 1996), and have generated a large body of data to both support and refine them. However, critique has focused on limitations in their explanatory power and developmental modelling. There are questions about the lack of specificity to autism of the Theory of Mind and the Executive Dysfunction hypotheses (Pennington and Ozonoff, 1996; Yirmiya et al., 1998). There are also questions about the degree of association between Theory of Mind skills and more general verbal and cognitive levels (Buitelaar et al., 1999), and the notion that the successful teaching of Theory of Mind skills does not necessarily lead to advancement in real-life social competence (Ozonoff and Miller, 1995). A weakness relevant to all the theories reviewed here is the lack of data connecting behavioural abnormalities, neuropsychological deficits, and brain substrates.

Behavioural treatment

Research suggests that early intervention can result in significant improvement for some children with autism, though for how many children and the amount of gain has varied considerably across studies (Rogers, 1998; Birnbrauer, 1997; Dawson and Osterling, 1997; McEachin et al., 1993; Harris et al., 1991; Lovaas, 1987). There is currently no medical or biological treatment of the core features of ASD. Thus, the primary source of intervention for most children is through their families and through the educational system. Traditional 1-hour a week treatments for language, social skills or behaviour are rarely sufficient to produce generalisable improvements in core areas of ASD (Howlin, 1998). Areas of general agreement between the programs offered, include the use of highly structured approaches to teaching, intensive involvement with the child, and a general focus on "learning to learn" challenges, i.e., basic abilities that involve participating in and benefiting from instruction (National Research Council, 2001).

However, even for well-established treatments like Applied Behaviour Analysis (the most widely studied treatment method), the results from recent randomised control trials (Smith et al., 2000) have not been as dramatic as those initially reported (Lovaas and Smith, 1988). Various factors appear to be central to successful intervention programs; children with less classical autism may respond better than those with more strictly defined autism, and children with better cognitive abilities or higher levels of engagement may respond more positively (Drew et al., 2002; Siller and Sigman, 2002; Wolery and Garfinkle, 2002; Rogers, 2000). No single approach is the best for all individuals, or even across time for the same individual with ASD (Volkmar et al., 2004). In addition, not all children with ASD benefit from treatments addressing the same goals. For example, teaching a pre-verbal child five words may be different than teaching five more words to a child that knows five words already.

Pharmacological treatment

Pharmacological treatment of children, adolescents, and adults is common in clinical practice. As the neurochemical basis of autism remains unknown (see below), there is no pharmacotherapy based on the pathogenesis of the core social and communicative deficits. Therefore, pharmacotherapy is approached from a pragmatic perspective. There are medications that ameliorate certain aspects of behaviour or mood, such as activity level (Aman, 1996) or irritability (Arnold et al., 2000). One may consider prescribing medication to subjects with autism to target symptoms such as hyperactivity, aggression and self-injury, stereotypies and rigidity, and anxieties (Buitelaar and Willemsen-Swinkels, 2000). Such interventions may be of great benefit to children with autism and their families; both directly, relative to symptom reduction, and indirectly, in helping the child profit more from behavioural and educational interventions (Volkmar, 2001). When starting with medication, it is important to select appropriate targets of treatment and to monitor efficacy and side effects on a regular basis (Buitelaar and Willemsen-Swinkels, 2000).

Neuropathology

Several postmortem studies have highlighted areas of anatomical abnormality in the autistic brain (Palmen et al., 2004b). Consistent findings have been observed in the limbic system, cerebellum, and inferior olive. In the limbic system, the hippocampus, amygdala, and entorhinal cortex have shown small cell size and increased cell-packing density at all ages, suggesting a pattern consistent with a development that is cut short (Bauman and Kemper, 2005). Findings in the cerebellum have included significantly reduced numbers of Purkinje cells, primarily in the posterior inferior regions of the hemispheres (in fact, the presence of reduced numbers of Purkinje cells is the most reproducible pathological observation in the autopsied autistic brain). With a few exceptions, there has been an absence of glial hyperplasia (Bailey et al., 1998; Bauman and Kemper, 1996) suggesting that the cerebellar lesions were acquired early in development.
A different pattern of change has been noted in the vertical limb of the diagonal

band of Broca, cerebellar nuclei and inferior olive with plentiful and abnormally enlarged neurons in the brains of young autistic subjects, and in adult autistic brains, small, pale neurons that are reduced in number. These findings, combined with reported age-related changes in brain weight and volume, have raised the possibility that the neuropathology of autism may represent an on-going process (Bauman and Kemper, 2005). Furthermore, given the known close relationship of the olivary climbing fiber axons to the Purkinje cell dendrites (Holmes and Stewart, 1908), the preservation of the olivary neurons in the face of a significant reduction in Purkinje cell number strongly supports a prenatal origin for the cerebellar abnormalities (Bauman and Kemper, 2005).

Neurochemistry

Current interest in the neurochemistry of autism is focused foremost on serotonin systems, as studies of the dopaminergic and noradrenergic systems in autism have failed to reveal consistent abnormalities (Buitelaar and Willemsen-Swinkels, 2000). One of the most robust and best-replicated findings in the neurobiology of autism is the elevation of the concentration of serotonin in whole blood of individuals with autism compared to normal controls (Anderson et al., 1990; Cook et al., 1990; Minderaa et al., 1989). In typically developing children, there is a period of high brain serotonin synthesis capacity during childhood. In children with autism, this developmental process is disrupted (Chugani et al., 1999). Serotonin is particularly interesting as serotonergic neurons are widely distributed throughout the brain and the system is one of the earliest to develop, and as such plays a critical role as a growth factor in the immature brain directing both proliferation and maturation (McDougle et al., 2005).

Brain metabolite concentrations

Magnetic resonance spectroscopy (MRS) is a technique that can be used to assess metabolite concentrations. Unlike magnetic resonance imaging (MRI), which provides high-resolution images of brain anatomy primarily using signals from brain water and lipids, MRS actually suppresses these high background signals in

order to measure the major brain neurotransmitters and metabolites (Moore, 1998). Each of the individual metabolites can be identified by its peak position expressed in chemical shift units: parts per million (ppm) (Moore, 1998). Spectroscopy adds functional information to the structural information seen with standard MRI scanning (Potts et al., 1993). When long echo times (>136 ms) are used, the most studied metabolites comprise N-acetyl-aspartate (NAA), choline (Cho), creatine (Cr) and lactate.

A reduction in NAA has been suggested to be indicative of reduced neuronal viability. However, mature oligodendrocytes can express NAA in vitro. This observation brings into question whether the NAA changes reflect neuronal function alone; there are also suggestions that NAA has a role in myelin maturation (Bhakoo and Pearce, 2000). Cho is found in cell membranes. It is a metabolic precursor and is increased during cell differentiation. Cho deficiency in cell cultures causes apoptosis or programmed cell death (Albright et al., 1996). Cr is a single resonance containing both creatine and phosphocreatine. Creatine is converted to phosphocreatine through the enzyme creatine kinase. Phosphocreatine is a high-energy phosphate that is critical for maintaining cellular energy dependent systems. The Cr peak has been used by a number of investigators as an internal standard for interpreting qualitative changes in the concentration of the other MR visible neurochemical compounds and as such, one often will see various references containing proton MRS data with Cr in the denominator (i.e., NAA/Cr or Cho/Cr). This reflects the assumption that the Cr resonance is relatively unaffected by various pathologies. This has yet to be definitively established and in several studies has, in fact, been reported not to be a valid assumption (Moore, 1998). The presence of lactate in a region of the brain is often associated with vascular (anaerobic) pathology and poor prognosis for the brain tissue involved.

In autism MRS research, it has been put forward that the field suffers from a lack of replication studies and poor methodology in terms of not controlling for confounding variables (Goldberg et al., 1999). In children with Asperger's syndrome significantly higher prefrontal concentration of NAA, Cr, and Cho have been reported. Here, prefrontal NAA concentration was significantly correlated

with the severity of obsessive/repetitive behaviour (Murphy et al., 2002). However, studies including individuals with autism and PDD-NOS report lower levels of NAA compared to typically developing (pre)adolescents, especially in the frontal and left frontoparietal white matter. Cho was reported to be lower in the left inferior anterior cingulate and Cr was higher in the right caudate head (O'Neill et al., 2002). In three- and four year old children with ASD. regional analyses demonstrated subtle patterns of reduced brain chemical alterations in ASD compared to typically developing and developmentally delayed children (Friedman et al., 2003).

Structural imaging

The proportion of macrocephaly is cited as approximately 20% of individuals with idiopathic autism (Lainhart, 2003; Fombonne, 1999). This is not solely an excess of megalencephaly among a minority of cases in a population with a normal mean head size. The increase in size appears to be a shifting of the entire autism brain and head size distribution toward larger heads and brains (Courchesne et al., 2003; Aylward et al., 2002; Fombonne et al., 1999). Head size does not appear to be increased at birth, but head circumference rather seems to accelerate in the first year of life (Courchesne et al., 2003; Lainhart et al., 1997). Several studies have reported accelerated head growth in children with ASD as young as one or two months old (Dementieva et al., 2005; Courchesne et al., 2003).

Recent findings suggest that the brain may be enlarged by as much as 10% in volume in toddlers with autism (Sparks et al., 2002; Courchesne et al., 2001). However, the magnitude of the effect appears to diminish with age. In adolescence and adulthood the effect is less consistently found across studies (Palmen et al., 2005; Palmen et al., 2004a; Herbert et al., 2003; Aylward et al., 2002; Courchesne et al., 2001; Piven et al., 1995), and the size of the effect is diminished to a few percent increase at most.
The regions of enlargement seem to be widespread (reviewed by Palmen and van Engeland, 2004; Brambilla et al., 2003), but may not present in all areas. For instance, Piven and colleagues (Piven et al., 1996) have reported increased volumes of the parietal, occipital, and temporal lobes in autism, whereas the frontal lobes

were normal. Among two- and three-year-old patients with autism, there appears to be an anterior-to-posterior gradient in volumetric enlargement (Carper et al., 2002). The frontal lobe exhibited the greatest degree of hyperplasia, while the occipital lobe volume was not significantly different from normal. Further, the group of Courchesne has reported a non-uniform enlargement of the frontal lobes in autism, with dorsolateral and medial frontal regions being hyperplastic in patients aged two to five years compared with control subjects of the same age; in contrast, the precentral gyrus and orbital cortex were not enlarged (Carper and Courchesne, 2005).

Increases in both grey and white matter have been reported in autism, with effect sizes varying as a function of the age of the cohort. In a cross-sectional study, increased cerebral grey matter volume (12% relative to normal, unadjusted for whole brain volume) was reported in young autistic children (two and three years old) whereas an older cohort of autistic children (12-16 years) had normal values (Courchesne et al., 2001). In contrast, Lotspeich and colleagues reported cerebral grey matter volume enlargement among subjects with autism relative to normal controls in a cohort that roughly overlapped the older cohort of Courchesne study (Lotspeich et al., 2004).

Furthermore, the Courchesne study reported an 18% increase in cerebral white matter in the younger cohort but normal values for the older cohort (12–16 years, Courchesne et al., 2001). The finding of increased cerebral white matter volume was replicated in a prepubertal cohort (7–11 years), with white matter volumes 15% greater than control volumes (Herbert et al., 2003). In this study, the white matter volumetric increase was confined to the radiate zone (the subcortical white matter primarily composed of the corona radiata and the U-fibers). Palmen et al. reported a larger grey matter volume in both children (Palmen et al., 2005) and adolescents (Palmen et al., 2004a). They reported no differences in white matter volume between autism and control groups. In both the autistic children and in the autistic adolescents, brain enlargement was global, and not restricted to a particular brain area. As such, at this point, it remains unclear whether the increase in brain volume is region specific, and whether it represents a change in predominantly grey or white matter.

There are several possible mechanisms for brain enlargement. There may be an over-production of cells, resulting from a decrease in apoptotic cell death, an increase in gliogenesis, which proceeds through postnatal development (Jacobson, 1991) or from neurogenesis. Still, a tight interrelationship exists between all pathways (Gould and McEwen, 1993). Other potential neurobiological mechanisms include an initial overgeneration of synapses, decreased synaptic pruning, abnormalities of myelin and excessive amount of neurotrophins (Nelson et al., 2001). At this point, there is no firm (pathological, morphologic or metabolic) evidence to support any of these suggested hypotheses.

Structural imaging of young children

To date, only three studies on autism have been published that were specifically aimed at children under the age of five (Hazlett et al., 2005; Sparks et al., 2002; Courchesne et al., 2001). The first published study (Courchesne et al., 2001) reported that by ages two to four years 90% of autistic boys had a brain volume larger than normal average and 37% met criteria for developmental macrencephaly (brain volume that exceeds the normal mean by two SDs). Autistic two- to three-year olds had more cerebral cortical grey matter (12%), and more cerebral (18%) and cerebellar white matter (39%) than typically developing controls. Sparks and colleagues (Sparks et al., 2002) also found significantly increased cerebral volumes in children with ASD compared to typically developing and developmentally delayed children. Cerebellar volume for the ASD group was increased in comparison to the typically developing group, but this increase was proportional to the overall increase in cerebral volume. The developmentally delayed group had smaller cerebellar volumes compared to the comparison groups. In the third study by Hazlett and colleagues, significant enlargement was detected in cerebral cortical volumes but not cerebellar volumes in individuals with autism. Enlargement was present in both grey and white matter, and it was generalised throughout the cerebral cortex (Hazlett et al., 2005). In the first two studies brain volume was reported to be approximately 10% larger in children with autism than in typically developing children (Sparks et al., 2002; Courchesne et al., 2001). In the third study, in which the children were slightly younger, this was approximately 5% (Hazlett et al., 2005).

The SOSO project

The studies described in this thesis were designed to be part of a larger study - the SOSO project – aimed at screening for ASD in very young children. SOSO stands for Screeningsonderzoek Sociale Ontwikkeling, or in English, Screening Study on Social Development. From October 1999 to April 2002, 31.724 children aged 14 to 15 months were first pre-screened by physicians at well-baby clinics using a four-item screening instrument (Dietz et al., in press; Willemsen-Swinkels et al., in press). A trained psychologist using the 14-item Early Screening of Autistic Traits (ESAT) then evaluated infants that screened positive during a 1.5-hour home visit. Children with three or more negative scores on the 14-item ESAT were considered to be at high-risk of developing ASD and were invited for further systematic psychiatric examination (Dietz et al., in press; Willemsen-Swinkels et al., in press). This included a series of five visits. During these visits, the social and emotional behaviour of the child was observed. The assessments also included a parental interview, developmental history, paediatric examination, and several developmental and diagnostic tests, e.g. the ADOS (Lord et al., 1989) and the Mullen Scales of Early Learning (Mullen, 1995). Based on all available information, a provisional diagnosis was made by an experienced child psychiatrist. The inter-rater reliability of the clinical diagnosis was calculated for two diagnostic categories: ASD or other than ASD. Agreement corrected for chance was 0.74 (Cohen's kappa). Diagnostic discrepancies were resolved at a consensus meeting. At age 42 months, the children were re-examined and received a final diagnosis. More details on the psychiatric diagnostic process will be reported elsewhere (van Daalen et al., in preparation).
Parents of children suspected of ASD or other developmental disorders were asked to participate in an extensive state of the art laboratory work-up, which included careful physical examination, and MRI and MR spectroscopy of the brain.

Aim and outline of this thesis

The overall aim of this thesis is to study the metabolic, radiologic, and volumetric brain correlates in very young children with severe developmental disorders; ASD, mental retardation, and language disorder. At the start of this project, no studies

specifically including very young children with ASD had been published. We wished to examine brain – behaviour relationships in children with ASD at a very young age. As part of the SOSO project, the studies in this thesis include a population-based cohort of children with ASD and children with other developmental disorders.

Are autism spectrum disorders at a very young age associated with radiological abnormalities of the brain?

In chapter 2, we examine the MR images of the participating children for the prevalence of brain abnormalities, such as cortical migration disorders, ventricular dilations, and disturbed myelination patterns. This is the second study of radiological abnormalities in autism and developmental delay (see also Piven et al., 1990) and the first to include children at a very young age.

Are autism spectrum disorders at a very young age associated with decreased neuronal integrity?

In chapter 3, the question of neuronal integrity in the frontal subcortical white matter and the amygdala-hippocampal complex is addressed using magnetic resonance spectroscopy. Based on previous literature, we hypothesise decreased neuronal integrity (as reflected in a lower level of N-acetyl-aspartate) in very young children with ASD, compared to neuronal integrity in children without ASD but with language disorder or mental retardation.

Is there a relationship between brain volumes and intelligence in children with developmental delay at very young age?

Chapter 4 takes a closer look at study participants diagnosed with mental retardation or language disorder. The relationship between intellectual functioning and brain volume has never been described in children with a developmental delay under the age of five. The relationship between brain volumes and intellectual functioning is investigated.

Are autism spectrum disorders at a very young age associated with differences in brain volumes?

The relationship between intellectual functioning and brain volume returns in chapter 5, where children with autism and PDD-NOS are also included. This chapter compares brain volumes between the ASD group and the developmentally delayed control group, and examines whether brain volumes are increased in children with ASD compared to brain volumes of children without ASD but with developmental delay. Based on previous studies, we hypothesise a larger total brain volume as well as a larger intracranial volume. This chapter also addresses the relationship of brain volumes and intellectual functioning in children with ASD.

Finally, in chapter 6 the findings of the studies are summarised and discussed. Furthermore, a general conclusion is presented and implications for future studies are discussed.

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2

Radiological findings in autistic and developmentally delayed children

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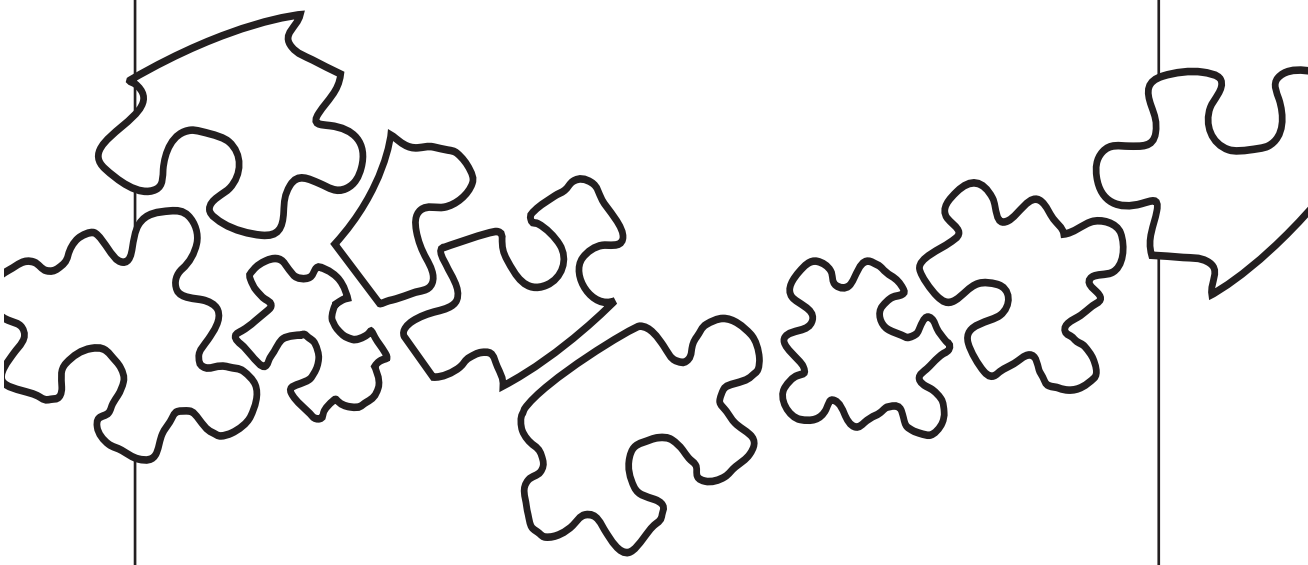
Brain and Development 28: 495-499



Abstract

Objective

The aim of this study was to evaluate the prevalence of brain abnormalities in a group of young children with developmental disorders, specifically including children that came to the attention of a child psychiatrist before the age of three years.

Methods

Forty-five children participated in an MR study (mean age 43 months, SD=12, four females). The study design was approved by the local Medical Ethical Review Board. All parents gave written informed consent. Two board-certified radiologists independently assessed scans for malformations of grey and white matter.

Results

Cohen's kappa for the consensus between the two raters was 0.79. In 22 children (49%), abnormalities were reported. Four patients (8.5%) had an arachnoid cyst. One female was diagnosed with a Chiari I malformation. Three children show enlarged Virchow-Robin spaces, an increased occurrence when compared to the normal population.

Conclusions

A high rate of intracranial abnormalities was found in this study. Radiological findings do not contribute to the diagnosis of developmental disorders. However, young children with developmental disorders may not be able to express discomfort associated with brain abnormalities, such as a Chiari I malformation. Given the high prevalence of abnormalities in this sample, neuroimaging may be a useful tool in clinically assessing children with developmental disorders.

Introduction

There have been several studies on malformations of cortical development in children with an abnormal cognitive or social development. Studies of patients with language disorders have reported polymicrogyria (Guerreiro et al., 2002) and white matter hyperintensities (Jernigan et al., 1991). Children with mental retardation were found to have enlarged Virchow-Robin spaces, cavum vergae and white matter hyperintensities (Soto-Ares et al., 2003). In children with autism spectrum disorders, there have been reports of accentuated Virchow-Robin spaces (Taber et al., 2004), acrocallosal syndrome (a rare autosomal recessive condition characterised by partial to complete agenesis of the corpus callosum, polydactyly, minor cranial dysmorphisms and mental retardation (Steiner et al., 2004; Skjeldal et al., 1998)), white matter hyperintensities (Courchesne et al., 2001), pachygyria (Schifter et al., 1994) and of macrogyria and polymicrogyria (Piven et al., 1990). Within autism spectrum disorders, the reported findings are diverse in both the nature of the abnormality and the incidence of abnormalities reported, ranging from 0% (Hardan et al., 2003) to 100% (Danielsson et al., 2002). This may be explained by the inclusion of different patient groups in the studies. Age, diagnosis (autism, PDD-NOS or Asperger syndrome), and co-morbidities such as epilepsy may have an influence on the number and type of abnormality found. Furthermore, there are differences in the nature of the studies, where some are designed to identify even the smallest brain malformations noticeable on MRI, whereas others only report large malformations that are observed in a volumetric study.

There have been discussions about the clinical relevance of MR scanning in children with a developmental delay or autism. MRI is judged to be of little value in the clinical diagnosis of autism according to the guidelines of the American Academy of Neurology and Child Neurology Society (Filipek et al., 2000). However, children who come to the attention of clinical psychiatrists at an early age may differ from children that are not seen until school age or preadolescence, who are often included in MR studies. The first group of children may show behaviour that is more deviant and have a lower level of functioning than the latter group (Lord,

1995). Therefore, children who are identified with developmental disorders at an early age may also differ in the amount or severity of brain abnormalities compared to children that were identified at a later age.
The aim of this study was to evaluate the prevalence of brain abnormalities in a group of young children with abnormal development, specifically including children that came to the attention of a child psychiatrist before the age of three years.

Materials and methods

Study design was approved by the Medical Ethical Review Board at the University Medical Center Utrecht. Participants were recruited from referrals to the Department of Child and Adolescent Psychiatry of the University Medical Center Utrecht. Diagnosis was made by a team of board certified child psychiatrists according to DSM-IV criteria (American Psychiatric Association, 1994). For children younger than 42 months at the time of scanning, a final diagnosis was made when they reached 42 months. Parents of children diagnosed with autism or PDD-NOS with or without concurrent developmental retardation, mental retardation (without an autism spectrum disorder) or language disorder were given the opportunity to participate in an MR scan. Patients were included if (a) they were 18 months to seven years of age, (b) were seen at the Child Psychiatry department before the age of three years for developmental disorders and subsequently diagnosed with an autism spectrum disorder, a language disorder or mental retardation, and (c) had no contraindication for anaesthesia or MRI. For all four groups (autism, PDD-NOS, mental retardation or language disorder), children having significant motor or sensory impairment (e.g., blindness, deafness), major physical abnormalities, history of serious head injury, identifiable neurological disorder or metal implants such as prostheses were excluded. Of the 45 children that were included in the study, 20 children were diagnosed with autism, 12 with PDD-NOS, four children were diagnosed with mental retardation, and nine with language disorder (mean age 43 months, SD=12, range 21-77 months). All parents gave written informed consent.

To measure developmental age, 19 children were administered the Mullen Scales of Early learning (Mullen, 1995). The Psychoeducational Profile-revised, PEP-R (Schopler et al., 1990) was used to assess the developmental age for ten children and for six children either the Griffith (Griffith, 1986) (in one case), the Kaufmann-ABC (Kaufmann and Kaufmann, 1983) (for two patients) or the Dutch nonverbal intelligence test, the SON-R (Tellegen et al., 1996) (for three patients) was used. Ten children were not able to complete any of the offered tests, due to lack of cooperation or anxiety. In those cases, the psychiatrist made clinical judgment of level of functioning. Level of developmental functioning was defined to four groups; 49 or below (moderately retarded), between 50 and 70 (mildly retarded), between 71 and 84 (borderline intellectual functioning) and 85 or higher (average functioning).

MR scans were made while patients were still under full anaesthesia following a lumbar puncture procedure. The following scans were acquired on a Philips NT Gyroscan scanner, operating at 1.5 Tesla; a 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, TR=2200), a T1-weighted three-dimensional fast field echo with 150 1.5-mm contiguous coronal slices of the whole head (TE=4.6 ms, TR=30 ms), and a T2-weighted dual echo turbo spin-echo with 75 3.0-mm contiguous coronal slices (TE1=14 ms, TE2=80 ms, TR 6.350 ms).

Two board-certified radiologists (RJN, TW) independently reviewed the scans. Both raters were blind for clinical, pathological, and other radiological reports. Each of the four scans acquired was rated for malformations of grey and white matter. Special attention was paid to the basal ganglia, the corpus callosum, the ventricles, the brain stem, the cerebellum, and the possible presence of a cavum septum pellucidum. Scans were also inspected for age-appropriate meylination. A meeting was held to reach consensus when inconsistencies were found between the reports of the two raters. All analyses were conducted using the SPSS statistical package (version 11.5). Categorization of the consensus is based on the method described by Landis and Koch (Landis and Koch, 1977).

Results

Patient information and radiological findings are listed in table 1.

The consensus of the radiologists' findings was 0.79 (Cohen's kappa), which is considered very good (Landis and Koch, 1977). 51% of the MRI scans had no abnormalities (23 patients). In 22 children (49%), abnormalities were reported.
Four patients were found to have a cyst. In three children, this was located in the left temporal arachnoid space (see figure 1). One child had a small right retrocerebellar arachnoid cyst. Three children (6.7%) were found to have wide perivascular spaces, also known as Virchow-Robin spaces. In one child (diagnosed with language disorder) this was found bilaterally, in the other two the perivascular spaces were more numerous either on the left or the right (both diagnosed with autism).
White matter abnormalities were reported in four children. White matter lesions were found in three children, mostly in the occipital lobe. One 21-month old female had the myelination pattern of a 10-month old child. Three children had either a shape variation or a thinning of the corpus callosum. Two children were found to have a cavum vergae. One of these children also had a Chiari I malformation (see figure 2).

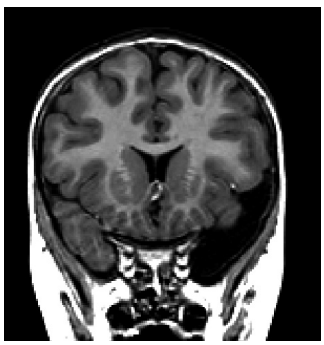


Figure 1
One of the three children with a arachnoid cyst located in the middle cranial fossa. This child was diagnosed with language disorder.

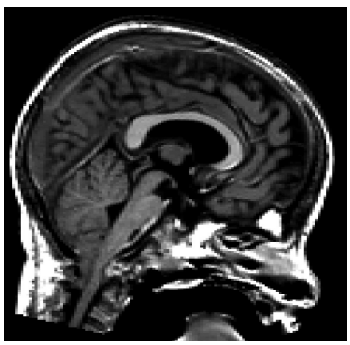


Figure 2
Female patient number 22 with a Chiari I malformation. This child was diagnosed with PDD-NOS.

Patient number	Clinical diagnosis	Sex	Age in months	Intellectual functioning	Radiological findings
1	A	M	50	moderate	Wide ventricles
2	A	M	46	borderline	White matter lesion
3	A	M	48	mild	
4	A	M	48	moderate	Wide ventricles
5	A	M	52	moderate	
6	A	M	35	moderate	
7	A	M	34	moderate	
8	A	M	55	moderate	Corpus callosum shape variation
9	A	F	49	moderate	Slight vermis atrophy
10	A	M	37	moderate	
11	A	F	21	moderate	Delay in myelination of 11 months
12	A	M	44	moderate	Wide frontal CSF spaces, bilateral wide occipital horns, bilateral occipital white matter lesions, occipital white matter loss, thinning of corpus callosum
13	A	M	33	borderline	
14	A	M	47	borderline	
15	A	M	27	moderate	
16	A	M	43	borderline	Asymmetrical wide Virchow-Robin spaces, most on the right
17	A	M	77	mild	Asymmetrical ventricles
18	A	M	41	borderline	
19	A	M	53	mild	Asymmetrical wide Virchow-Robin spaces, most on the left
20	A	M	45	moderate	
21	NOS	M	48	average	
22	NOS	F	33	average	Chiari I malformation, cavum septum pellicidum and vergae
23	NOS	M	36	average	
24	NOS	M	70	mild	Corpus callosum shape variation
25	NOS	M	38	borderline	Cavum septum vergae
26	NOS	M	37	mild	

Patient number	Clinical diagnosis	Sex	Age in months	Intellectual functioning	Radiological findings
27	NOS	M	49	average	
28	NOS	M	28	borderline	White matter lesions around occipital horns, thereby slightly angular occipital horns, possible perinatal asphyxia
29	NOS	M	54	moderate	
30	NOS	M	49	average	
31	NOS	M	50	average	
32	NOS	M	52	average	
33	LD	M	26	average	Left temporal arachnoid cyst
34	LD	M	29	mild	
35	LD	M	27	mild	Transcortical veins
36	LD	M	33	mild	Bilateral wide Virchow-Robin spaces
37	LD	M	32	average	
38	LD	M	42	average	
39	LD	M	42	mild	Small right retrocerebellar arachnoid cyst
40	LD	M	62	average	Mega cisterna magna
41	LD	M	50	mild	Left temporal arachnoid cyst
42	MR	F	61	moderate	Mega cisterna magna, left temporal arachnoid cyst, wide ventricles
43	MR	M	31	borderline	
44	MR	M	46	mild	
45	MR	M	35	borderline	Thinning of the corpus callosum, hypoplasia of the falx

Table 1 *Clinical and radiological results.*

A = autism, NOS = pervasive developmental disorder, not otherwise specified, LD = language disorder, MR = mental retardation, M = male, F = female, severe = (estimated) level of cognitive functioning 49 or below, mild = (estimated) level of cognitive functioning between 50 and 70, moderate = (estimated) level of cognitive functioning between 71 and 84 and average = (estimated) level of cognitive functioning 85 or higher.

Discussion

We report that in a group of young children with developmental disorders, 49% of the patients also had intracranial abnormalities on MRI. In 8.5% of the patients in this study, an arachnoid cyst was identified. In 75% of cases, this was located in the middle cranial fossa. A marked preponderance of middle fossa cysts for the left side has been reported (Wester, 1999), as was found in our three patients. In a study of incidental findings in 1000 asymptomatic adult volunteers, a 0.3% incidence of arachnoid cysts was found (Katzman et al., 1999). In a comparable study including 225 healthy children, only two children (0.09%) were found with an arachnoid cyst (Kim et al., 2002). We find the incidence of arachnoid cysts markedly increased in children with developmental disorders.

Recently, Taber and colleagues reported dilated Virchow-Robin spaces in 44% of children with autism between the ages of 7 and 18 (Taber et al., 2004). Virchow-Robin spaces are fluid containing dilatations of the perivascular space that surround penetrating arteries of the brain. In adults, they are associated with advancing age and hypertension. In our study, we have found dilated Virchow-Robin spaces in three patients (6.7%). Two of these children were diagnosed with autism, whereas the third was diagnosed with language disorder. The incidence of enlarged Virchow-Robin spaces in children is normally low (Heier et al., 1989). Therefore, the higher incidence of enlarged Virchow-Robin spaces in children with autism or a developmental delay may have clinical significance (Taber et al., 2004).

Reported percentages of radiological findings in patients with developmental disorders range from 0% to 100%. The latter study included children with epilepsy, which is often caused by cortical malformations (Danielsson et al., 2002). The first finding was reported in a study of 40 high functioning patients with autism between the ages of 8 and 45. In language disorder the reports vary between 20% (Jernigan et al., 1991) and 100% (Guerreiro et al., 2002). These inconsistencies may be attributed to the populations studied; possibly different subgroups, with different levels of functioning, presence or absence of epilepsy or to the method of assessing abnormalities (what are the regions of interest? What is considered a deviation from normal?).

This study included children that were seen early in development, and as such, these children may be a subgroup of patients with a more severe developmental disorder (Smeeth et al., 2004). This may explain why more intracranial abnormalities were found in this study, compared to 0% in studies of higher functioning patients (Hardan et al., 2003), who are often identified at a later age (Smeeth et al., 2004). Similarly, a selection bias may have contributed to the current finding, as families with the more severely affected children may be more likely to want their children to have MR scans (Miles and Hillman, 2000).

There have been discussions about the clinical relevance of MR scanning in children with a developmental delay or autism. The yield of MRI has been suggested to be low, as most findings are viewed to be incidental (Kosinovsky et al., 2005). However, in this study one patient was diagnosed with a Chiari I malformation and four children were found to have arachnoid cysts. Both malformations can give rise to severe headaches. Developmentally delayed children and children with autism may not be able to express pain satisfactorily, due to problems in verbal communication, body representation and cognition (Tordjman et al., 1999). A MR diagnosis of a brain malformation that may induce pain can alert parents and clinicians to physical discomfort that may not be fully expressed by the child.

This study focused on radiological findings in very young children with developmental disorders. We found radiological abnormalities in 49% of these children. MR scans do not have direct additional value for the diagnosis of autism or other developmental disorders, but some findings (e.g. Chiari I malformations, intracranial cysts) may contribute to clinical assessment of individuals with developmental disorders.

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3

Proton magnetic resonance spectroscopy in developmentally delayed young boys with or without autism

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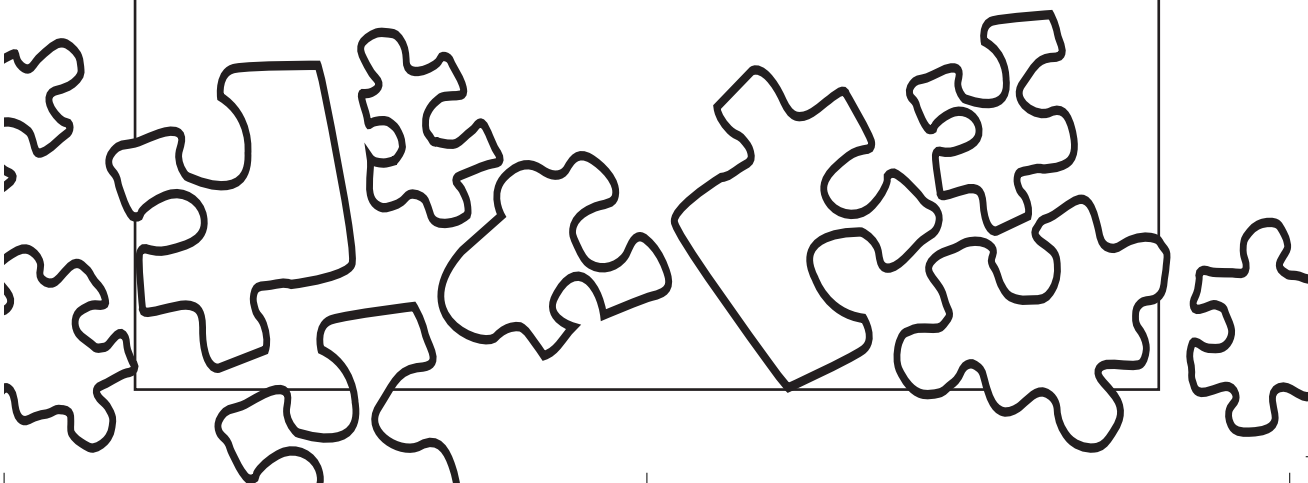
Journal of Neural Transmission, in press



Abstract

Objective

The aim of the present study is to investigate whether brain metabolism of boys with autism spectrum disorder is altered compared to boys with a developmental delay without autism if corrected for patient age and developmental level.

Methods

25 boys with ASD (with or without concurrent mental retardation) and 12 boys without ASD with mental retardation or language disorder underwent proton magnetic resonance spectroscopy. All analyses were performed with chronological age and developmental level as independent variables.

Results

No metabolic differences were found between boys with ASD and without ASD.

Conclusions

Our findings do not replicate previous reports of differences in NAA, Cho and Cr levels in ASD.

Introduction

Autism is a pervasive developmental disorder characterised by a triad of social deficits, language and communication problems and a pattern of stereotyped, repetitive and restricted behaviours and interests (American Psychiatric Association, 1994). It is a clinically heterogeneous condition that has a broad range of severity and is frequently associated with concomitant learning disability.

Imaging of patients with autism offers valuable information on anatomy and function (Akshoomoff et al., 2002; Cody et al., 2002). At present, several magnetic resonance spectroscopy (MRS) studies in patients with autism (in the age range of 2–32 years) have been performed. In general, a trend of decreased N-acetylaspartate (NAA) concentrations, decreased NAA/choline (NAA/Cho) or decreased NAA/creatine (NAA/Cr) ratios has been reported (Friedman et al., 2003; O'Neill et al., 2002). Still, interpretation of MRS data in autism studies is complicated. In addition to differences in absolute and relative metabolic concentrations between studies, regions of interest vary widely (Rumsey and Ernst, 2000; Sokol et al., 2002; Yurgelun-Todd and Renshaw, 2000). Otsuka et al. found lower NAA levels in the amygdala-hippocampal region (Otsuka et al., 1999), whereas others made similar observations in the cerebellum (Chugani et al., 1999) or parietal cortex (Hashimoto et al., 1997). More important, in general, it is difficult to elucidate which cerebral metabolic changes are related to autism itself. Approximately three-quarter of the patients with autism also suffers from learning disabilities, which in itself may give rise to metabolic abnormalities (Volkmar and Pauls, 2003). Therefore, when comparing a group of (retarded) autistic children with a group of normal developing children (Hashimoto et al., 1995; O'Neill et al., 2002), autism itself as well as mental retardation may underlie metabolic differences. Except for a study of patients with Asperger syndrome (Murphy et al., 2002), none of the MRS studies in this field used a correction for mental retardation. Moreover, the effects of age dependency on metabolic concentrations are often underestimated. Developmental studies have shown that the concentration of NAA, Cr, and glutamine and glutamate increases with brain development, whereas the concentration of Cho and myo-inositol decreases with brain development (Bhakoo and Pearce, 2000; Friedman et al., 2003; Kreis et al., 1993; Moore, 1998). In this respect, it is

important to keep the age range of both patient and (age matched) control group as small as possible. Even then, including age as an independent covariate in data analysis seems obligatory.

The overall purpose of the present study is to replicate the previously reported abnormalities in our group of boys with ASD and a control group of boys with a developmental delay without ASD. The aim was to determine these possible metabolic cerebral changes in the amygdala-hippocampal region and in the frontal subcortical white matter. All participants were between the ages of 1 year 9 months and 6 years 7 months. To correct for the effects of age and developmental level, all analyses were performed with chronological age and developmental level as independent variables.

Material and methods

Thirty-seven children participated. Participants were recruited from referrals to the Department of Child and Adolescent Psychiatry of University Medical Center Utrecht. Diagnosis was made by a team of board certified child psychiatrists (EvD, JB, HvE) according to DSM-IV criteria (American Psychiatric Association, 1994). Children younger than 42 months at the time of scanning had a final diagnosis when they reached 42 months. The diagnosis was confirmed by Autism Diagnostic Interview Revised (Lord et al., 1994) and the Autism Diagnostic Observation Schedule G (Lord et al., 1989) diagnoses.
When a child was diagnosed with autism or PDD-NOS with or without concurrent developmental retardation, mental retardation (without an autism spectrum diagnosis), or language disorder, parents were offered the possibility of MRI and MRS. Patients were included if a) they were 18 months to 7 years of age, b) were diagnosed with an autism spectrum disorder, language disorder or mental retardation, and c) had no contraindication for MRI. For all four groups, children having significant motor or sensory impairment (e.g., blindness, deafness), major physical abnormalities, history of serious head injury, identifiable neurologic disorder or metal implants such as prostheses were excluded. Patients were scanned under full anaesthesia with sevofluran. The study design was approved by the Medical

Ethical Review Board of the University Medical Center Utrecht. All parents gave written informed consent.

Children were administered the Mullen Scales of Early Learning (Mullen, 1995) to measure developmental level. Several children were at floor of the standardised scores. It was decided, therefore, to convert raw scores to a developmental level in order to allow us to look more closely at the functioning of the more impaired children. Four subtests were used; visual receptive, fine motor, receptive language, and expressive language. The developmental level was calculated as Mean Age Equivalent of the four subtests/Chronological Age x 100. For some children the Mullen was found to be too difficult. For seven patients with autism and one patient with PDD-NOS the Psychoeducational Profile – Revised (Schopler et al., 1990) was used to assess the developmental level. For five children either the Griffith (Griffith, 1986) (in one case with PDD-NOS), the Dutch Snijders-Oomen niet-verbale intelligentietest (Tellegen et al., 1996) (for one patient with language disorder and one with autism) or the Kaufman Assessment Battery for Children (Kaufmann and Kaufmann, 1983) (for two patients with PDD-NOS) was used.

The group with either autism or PDD-NOS, the ASD group, included 25 children (17 were diagnosed with autism, eight with PDD-NOS). The control group, with mental retardation or language disorder, included 12 children (four children were diagnosed with mental retardation, eight with language disorder). See table 1 for further descriptives of the sample. The groups differed on mean developmental level ($p<0.005$), but not on chronological age.

	Autism	PDD-NOS	Mental retardation	Language disorder
N	17	8	4	8
Age in months (SD)	43 (7)	45 (15)	40 (8)	39 (14)
Developmental quotient (SD)	44 (8)	80 (13)	67 (10)	86 (8)

Table 1 *The VOI in the amygdala-hippocampal complex*

Participants had a physical examination and medical history, including assessment of perinatal circumstances and maternal illness during pregnancy, completed by a developmental paediatrician. An audiologist and speech and language therapist performed hearing and language evaluations. During admission on the neuropaediatric ward of the hospital, extensive blood work, including amino acids, thyroid function, a karyogram and Fragile X testing was completed (van Daalen et al., in preparation). Two children were born prematurely at thirty and thirty-three weeks of gestation with weights accordingly. All children were born with a birth weight above fifteen hundred gram. No children were diagnosed with a metabolic disorder. All children had a karyogram as expected according to gender and no children tested positive for Fragile X or 22q11 deletion syndrome. Two children were treated with valproic acid for symptoms of epilepsy; one boy with autism, no mental retardation and one boy with mental retardation. Two children used thioridazine for excessive fear and panic attacks during which they displayed self-destructive behaviour.

All scans were acquired on a Philips NT Gyroscan scanner, operating at 1.5 Tesla. The proton MRS investigations were performed with a single voxel technique. Two volumes-of-interest (VOI) were selected, one in the frontal subcortical white matter (figure 1) and another in the amygdala-hippocampus complex (figure 2) of each subject. These regions are implicated by neuropathological studies (Araghi-Niknam and Fatemi, 2003; Kemper and Bauman, 1998). Both VOIs were placed in the left hemisphere. This hemisphere was chosen based on studies that found a significantly greater left than right hemisphere dysfunction (Chiron et al., 1995; Dawson, 1983).

The VOIs were chosen from a 3D-FFE T1 (TR/TE=30/4.6 ms, FOV=256/70%, matrix 256x256, slice thickness=1.5 mm, flip=30). The dimensions of the selected VOIs were typically 15 mm in anterior-posterior, 10 mm in left-right directions and 10 mm in caudo-cranial directions. Special care was taken to position the VOIs away from grey matter (in the case of the frontal subcortical white matter voxel) and CSF and blood vessels (in the case of the amygdala-hippocampal voxel) and the VOI size was reduced when necessary. After selection of a VOI, the 90-degree pulse length was determined. To minimise eddy currents and to maximise the water echo

signal, localised spectroscopy was first performed without water suppression for adjustments of the gradients ('gradient tuning'). This was followed by localised automatic shimming of the VOI, resulting typically in a water resonance line-width of 6 Hz (full width at half maximum) or less. Water suppression was performed by selective excitation (60 Hz bandwidth), followed by a spoiler gradient. A double spin-echo PRESS (point resolved spectroscopy) sequence was used for VOI localisation. Each measurement was performed with a repetition time of 2000 ms, an echo time of 144 ms, 2048 time domain data points, 4000 Hz spectral width, and 64 signals acquired.

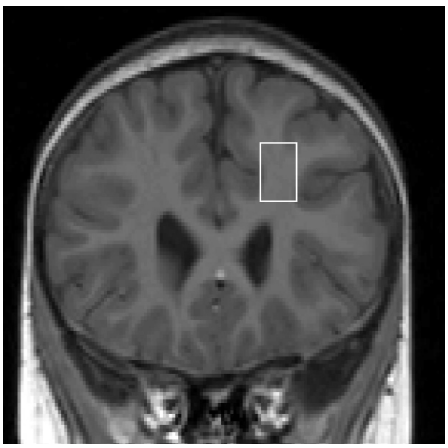


Figure 1 *The VOI in the frontal subcortical white matter*

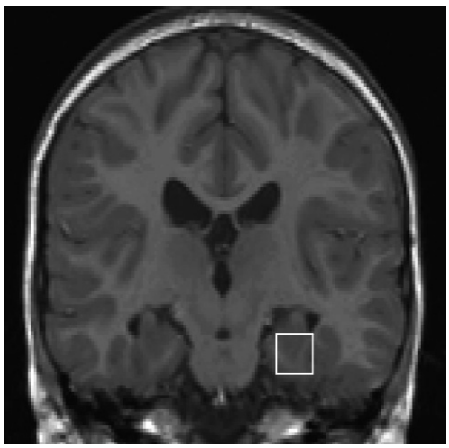


Figure 2 *The VOI in the amygdala-hippocampal complex*

After zero-filling to 4096 data points, exponential multiplication of 2 Hz, Fourier transformation and linear baseline correction, NAA (referenced at 2.0 ppm), total choline (referenced at 3.23 ppm) and total creatine (referenced at 3.02 ppm) peaks were quantitated with the program MRUI, using prior knowledge (van der Veen et al., 1988). Additionally, a water reference spectrum was obtained in order to calculate absolute concentrations of NAA, Cho, and Cr.

All analyses were conducted using the SPSS statistical package (version 9.0). We used logistic regression to compare metabolites and metabolite ratios in both VOIs in the autism and the control groups. In the logistic regression analyses, we adjusted for chronological age and developmental level.

Results

Typical examples of proton MR spectra of children in the ASD group and of children in the control group in the frontal subcortical white matter and the amygdala-hippocampal complex are shown in figure 3. Mean VOI size in the frontal -subcortical white matter was 1145 (SD=178). Mean VOI size in the amygdala-hippocampal complex was 1091 (SD=199).

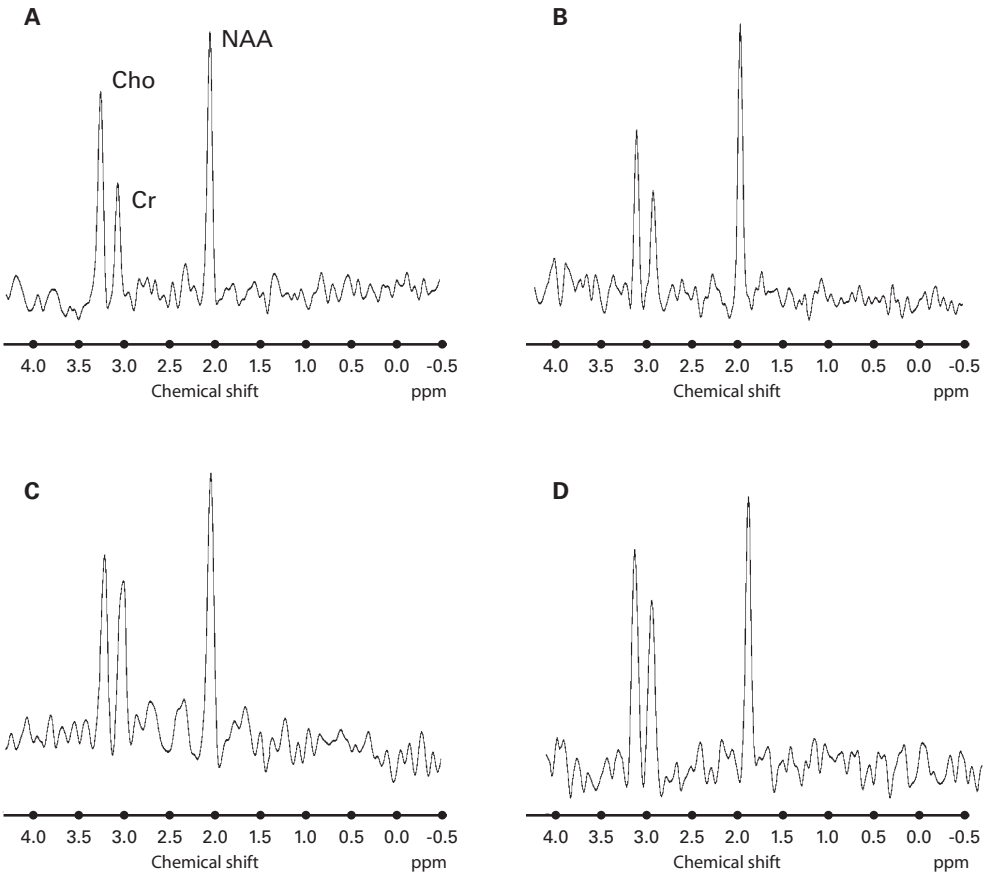


Figure 3 *Typical MR spectra*
3a: ASD group frontal, 3b: mental retardation group frontal
3c: ASD group amygdala, 3d: mental retardation group amygdala

Groups did not differ in mean VOI size. No differences in Cho, Cr, and NAA concentration nor in NAA/Cho and NAA/Cr ratios were found between the ASD group and the control groups in the frontal subcortical white matter (table 2). In addition, no metabolic differences were found between the ASD group and the mental retardation group and the language disorder group in the amygdala-hippocampal complex (table 3).

Prefrontal subcortical white matter					
	Cho	Cr	NAA	NAA/Cho	NAA/Cr
ASD (SD)	2.10 (0.57)	5.43 (1.09)	9.46 (1.29)	1.43 (0.42)	2.21 (0.45)
(N)	(24)	(25)	(24)	(24)	(24)
MR (SD)	1.75 (0.24)	5.01 (1.81)	9.21 (0.49)	1.58 (0.27)	2.54 (1.12)
(N)	(4)	(4)	(4)	(4)	(4)
LD (SD)	1.94 (0.83)	5.03 (1.31)	8.91 (1.58)	1.28 (0.37)	2.22 (0.39)
(N)	(8)	(8)	(8)	(7)	(8)

Table 2 *The metabolite concentrations in the frontal subcortical white matter, mean (SD). No significant differences were found. The N may be smaller than 37. Due to e.g. motion artefacts, some measurements were unusable. MR = mental retardation, LD = language disorder.*

Amygdala-hippocampal complex					
	Cho	Cr	NAA	NAA/Cho	NAA/Cr
ASD (SD)	1.48 (0.52)	5.73 (1.97)	7.06 (1.09)	1.53 (0.46)	1.63 (0.50)
(N)	(21)	(21)	(21)	(23)	(22)
MR (SD)	1.66 (0.48)	4.87 (1.40)	5.61 (0.19)	1.05 (0.34)	1.47 (0.47)
(N)	(2)	(2)	(2)	(2)	(2)
LD (SD)	1.45 (0.40)	5.15 (1.24)	6.57 (1.20)	1.42 (0.39)	1.63 (0.45)
(N)	(8)	(8)	(8)	(8)	(8)

Table 3 *The metabolite concentrations in the amygdala-hippocampal complex, mean (SD). No significant differences were found. The N may be smaller than 37. Due to e.g. motion artefacts, some measurements were unusable. MR = mental retardation, LD = language disorder.*

Moreover, in none of the subjects lactate resonances were found in any of the regions. Analyses were repeated without adjusting for chronological age and developmental level. Results did not change.

We did however find a significant positive correlation between age and Cr (r2=0.44, P=0.006) and age and NAA (r=0.57, P < 0.001) in the frontal subcortical white matter, indicating rising levels of Cr and NAA in the frontal cortex, as children get older (see figure 4).

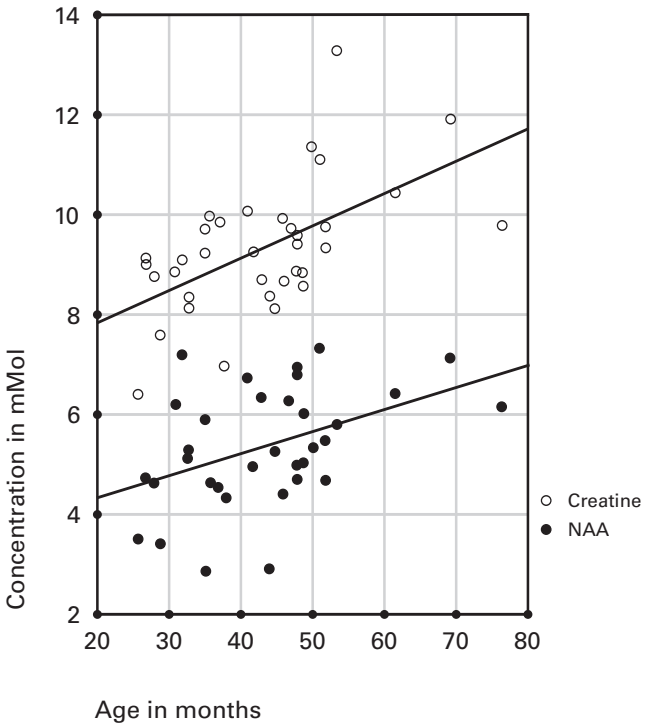


Figure 4 *The correlation of age with frontal NAA (r=0.57, P < 0.001) and frontal Cr (r=0.44, P=0.006)*

Discussion

The most important finding of this study is that, while controlling for chronological age and developmental level, we did not find any difference in NAA, Cho, and Cr concentration in the frontal lobe and in the amygdala-hippocampal complex between boys with ASD and boys with mental retardation or language disorder.
Previous studies have implied a decreased NAA level in some parts of the brain in autism (Chugani et al., 1999; Filippi et al., 2002; Friedman et al., 2003; Hashimoto et al., 1997; Murphy et al., 2002; O'Neill et al., 2002; Otsuka et al., 1999; Volkmar and Pauls, 2003). The NAA signal reflects tissue concentrations of both NAA and N-acetylaspartylglutamate. It has been suggested that the former may be an acetyl-group carrier between mitochondria and cytoplasm in neuronal cells (Kok et al., 2002). A decrease of NAA level is usually interpreted as a reduction in neuronal functioning. In our study, in which we corrected for chronological age and developmental level, we were not able to replicate these findings, indicating that no differences in brain development (neuronal functioning) are present between children with autism and children with a developmental delay in this age window. This implicates that between the ages of two and six years, children with autism or PDD-NOS do not have different cerebral metabolism when compared to children with either mental retardation or language disorder. One cannot rule out the possibility of differences appearing at a later age.

Our results confirm the results of a study by Hashimoto et al (Hashimoto et al., 1998). This study compares subjects with autism with typically developing children. In this study, the NAA/Cho, NAA/Cr and Cho/Cr ratios in the parietal lobe were not significantly different between the children with autism and control children. However, it should be noted that the study contained patients groups with a large age distribution (from four to 20 years), and data were analysed without any correction for chronological or developmental age, which might have led to negative results. In another study by the same group (Hashimoto et al., 1997), no differences were found between the autism group and the typically developing children. They did however report finding a lower NAA/Cho in the group with mental retardation compared to the ASD group and typically developing children. No

correction for chronological or developmental age was done. A recent study by Levitt et al. (Levitt et al., 2003) found no differences in NAA between the group patients with autism and the normal control group. However, they did find differences in Cho and Cr levels, especially in the caudate. The control group was matched to the autism group on chronological age (both groups included participants between 5 and 16 years), but not on developmental level.

As mentioned previously, most studies found slightly decreased NAA concentrations (Friedman et al., 2003; Levitt et al., 2003; O'Neill et al., 2002) or NAA related metabolic ratios (Hashimoto et al., 1997) in patients with autism. At the Human Brain Mapping meeting of 2002 O'Neill et al. presented a spectroscopic imaging, study comparing 28 ASD patients with 18 normal controls (O'Neill et al., 2002). They reported a decreased NAA in the frontal cortex and the left frontal-parietal white matter in the autistic group. They also reported decreased Cho levels in the left anterior cingulate gyrus and increased concentration of Cr in the right caudate head. However, groups were not matched for chronological or developmental age nor did analyses correct for these factors. Hisaoka et al. (Hisaoka et al., 2001) found a decreased NAA concentration in the temporal region in a group of 55 patients with autism, compared with a group of healthy controls. They included subjects between 0 and 21 years of age. No correction for age or developmental level was done. A previous study by the same group (Otsuka et al., 1999) included children between the age of two and 18 years and again there was no correction for chronological or developmental age related metabolic changes. Chugani et al. (Chugani et al., 1999) compared nine autistic children with five sibling controls without controlling for age or developmental level. Although all these studies found metabolic differences between probands and their control group, no correction for age or developmental level was done.

The effect of age on metabolite changes has been described extensively (Kreis et al., 1993). The predominant changes with brain development include an increase in concentration of NAA, Cr, and glutamine and glutamate, and a decreased concentration of Cho and myo-inositol (Moore, 1998). Looking at ratios, one sees an increase with age in NAA/Cho and NAA/Cr ratios; the Cho/Cr ratio decreases with

age in the majority of infants (Grattan-Smith et al., 1996). These latter two changes are thought to be associated with myelination of the developing brain (Moore, 1998). Even though the most rapid changes have been noted during the first three years of life, changes have still been observed up to the age of 16 years (van der Knaap et al., 1990). Our finding of a correlation of Cr and NAA with age in the frontal cortex confirms these age-related changes also in children with an abnormal development, either with or without autism.

In addition to age differences, stage of development has a large effect on metabolite levels as well. Filippi et al. (Filippi et al., 2002) found that children with a developmental delay older than two years had a decreased NAA/Cr ratio in the frontal and parieto-occipital subcortical white matter compared with paediatric controls (children who were scanned for headache, migraine, or epilepsy, but whose MRI was considered normal). In a recent study, children with ASD were compared with a group of normal controls and a group of children with a developmental delay without autism (Friedman et al., 2003). It was found that ASD subjects demonstrated reduced levels of NAA, Cr, and myo-inositol concentrations compared to typically developing children. There were no differences in Cho and Cr between ASD and developmental delay in this study. Nor were there differences in NAA levels in the frontal, temporal or parietal cortex. Only a slight decrease in occipital NAA was found in the ASD group.

There are several limitations of our study. Although no statistical differences between the groups were found, this could have been due to a type II error. However, a power analysis, calculated for NAA in the frontal voxel (a=0.05, b=0.10) shows that at least 537 samples in each group would have been necessary to find a significant difference.

Secondly, our group of patients with ASD included a large proportion of children with a low level of functioning. It is possible that there are differences in the metabolite concentrations between high- and low-functioning groups. A study by Jung et al. (Jung et al., 1999) found that in normal adults, NAA and Cho were independently associated with full-scale IQ. Moreover, the only study in the field of autism research to find a higher level of NAA compared patients with Asperger syndrome to healthy comparison subjects (Murphy et al., 2002). However, we believe that correcting for developmental level assures that the results of the study can be

generalised to the entire population of patients with autism. Unfortunately, several tests were used to measure the level of cognitive functioning of the boys in this study. Underlying this are problems with test taking due to low levels of functioning or anxiety, that are inherent to these populations. However, we believe that results of this study would have been no different had patients been tested with the same developmental scales.

It cannot be excluded that differences may have been detected, had measurements been done in other anatomic areas. Still, the frontal cortex and the amygdala-hippocampal region have often been implicated as involved in autism (Araghi-Niknam and Fatemi, 2003; Kemper and Bauman, 1998). Sampling was done only in the left hemisphere, which differs from the bilateral sampling done in most studies. This may account the lack of differences. However, based on studies that found a greater left than right hemisphere dysfunction (Chiron et al., 1995; Dawson, 1983), it is not expected that right hemispheric sampling would have shown group differences.

Fourth, we did not measure relaxation times as did Friedman et al. (Friedman et al., 2003), who reported a prolonged T2 relaxation of NAA for the autism group compared to the typically developing and developmental delay groups. Relaxation times are indicative of neuronal density, but they increase scanning time substantially, which in turn increases the strain on the children.

Furthermore, scans were made under full anaesthesia. This could not have influenced the results of this study, since all children received the same anaesthesia. It might however have implications for the comparison of our results to those of other studies. In the study of Friedman et al. (Friedman et al., 2003) the autistic group and the mental retardation group were scanned under anaesthesia, but not the typically developing children. This might account for differences reported between these groups.

We found that the outcome of our study did not change without correcting for chronological age or developmental level. This study only included children within a small age range. The effect of correcting for age is very small because of the small age range in our study. Still, we believe that using both developmental level and chronological age in the calculations is essential in child psychiatric research.

To conclude, in this study, we measured NAA, Cho, and Cr in a group of young boys with autism and a control group of boys with a developmental delay without autism. When controlling for chronological age and developmental level, we have found no differences between these groups.

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4

Intellectual functioning is related to brain volume in young children with developmental delay

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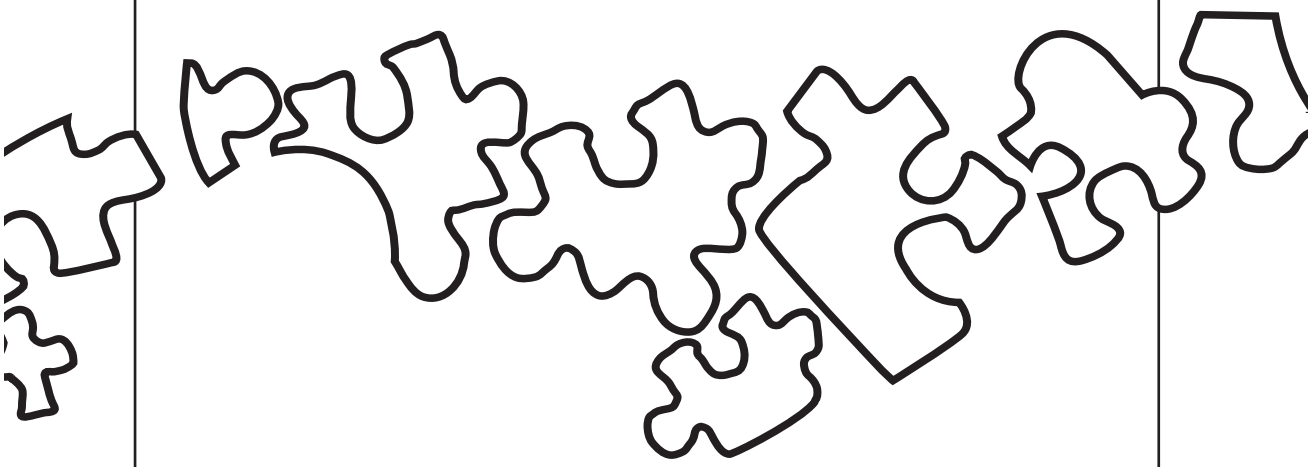

Abstract

Objective

Brain size is a modest predictor of intellectual ability in adults and (pre-)adolescent children. This study investigated the relationship between intellectual functioning and brain volume in children with a developmental delay under the age of five years.

Methods

Intellectual functioning, intracranial volume, total brain volume, and ventricular volume were assessed in 13 children. Pearson correlation coefficients were calculated.

Results

There was a significant correlation of 0.74 between intellectual functioning and total brain volume, after controlling for intracranial volume. A significant negative correlation was found between intellectual functioning and total ventricular volume (r=-0.77). There was no correlation between intellectual functioning and total brain volume or intracranial volume.

Conclusions

These results suggest that there may be relative brain volume loss related to poor intellectual functioning in young children with idiopathic developmental delay.

Introduction

Several studies have shown that overall brain volume is associated with general cognitive functioning in humans. In adults, moderate correlations of 0.20 to 0.40 have been reported (Andreasen et al., 1993; Toulopoulou et al., 2004), and in (pre-)adolescent children reported correlations range between 0.20 and 0.28 (Reiss et al., 1996; Wilke et al., 2003). In a very recent study, it was demonstrated that the level of intelligence was related to the pattern of cortical growth in childhood and adolescence, indicating that the neuroanatomical expression of intelligence in children is dynamic (Shaw et al., 2006). The first years of life are thought to be pivotal in the development of both the brain and intellectual functioning. However, it is unclear whether cognitive functioning and brain volume are associated in children under the age of five. Some studies have taken head circumference as an indicator of brain size in young children (Bartholomeusz et al., 2002). In children small for gestational age, a small head circumference has been associated with lower intellectual functioning (Frisk et al., 2002). However, head circumference is a relatively crude measure of brain volume. Therefore, we used high-resolution magnetic resonance brain imaging to investigate the relationship between intellectual functioning and brain volume in children younger than five years of age.

Methods

Thirteen children with developmental delay were included; five children had a diagnosis of mental retardation and eight children were diagnosed with language disorder according to the definitions of DSM-IV (mean age 41 months, SD=12, range 26-62 months). Intellectual functioning was operationalised as the non-verbal intelligence measure (the fine motor and visual acuity subtests) of the Mullen Scales of Early learning (Mullen, 1995). Verbal measures were not taken into account as eight children had been diagnosed with language disorder and non-verbal tests are considered more suitable for children with mental retardation (Skovgaard et al., 2004). The developmental quotient was calculated as the mean of the age equivalents of the two non-verbal subtests divided by chronological age times 100, representing a position in a normal distribution with a given mean of

100 and a given standard deviation of 15 (Mullen, 1995). In one case, a Dutch non-verbal intelligence test, the SON-R (Tellegen et al., 1996), comparable to the Mullen scales, was used. All statistical analyses were repeated without this child.
All participants completed an extensive medical assessment. A developmental paediatrician performed a physical examination and medical history, including assessment of perinatal circumstances and maternal illness during pregnancy. An audiologist and speech and language therapist evaluated hearing and language. All subjects were admitted to the neuropaediatric ward of the UMC Utrecht for an extensive blood screening, including amino acids, thyroid function, a karyogram, Fragile X testing and screening for metabolic disorders. The admission included consults by a paediatrician, child neurologist, and clinical geneticist. A lumbar puncture procedure was performed to assess the presence of metabolic abnormalities (van Daalen et al., in preparation). No metabolic or genetic abnormalities were reported for any of the children included in the current sample. All children had a karyogram as expected according to gender. Two children were born prematurely at thirty-three and thirty-four weeks of gestation with accordingly lower birth weights. All children were born with a birth weight above fifteen hundred grams. One boy diagnosed with mental retardation had been previously treated with valproic acid for symptoms of epilepsy. No birth complications were reported for any of the subjects. As such, the sample included in the current report is representative of children with idiopathic developmental disability not due to an detected biological cause. A control group of typically developing children could not be included as the children were below the allowed age for inclusion in MR studies for research purposes, as stated by the Dutch Central Committee on Research involving Human Subjects.

T1-weighted three-dimensional fast field echo scans with 1.5-mm contiguous coronal slices of the whole head and T2-weighted dual echo turbo spin-echo scans with 3.0-mm contiguous coronal slices were acquired on a Philips Gyroscan (Philips Medical Systems, Best, The Netherlands) at 1.5 Tesla. The MRI procedure was planned after the lumbar puncture procedure, while patients were still under full anaesthesia. The study design was approved by the Medical Ethical Review Board of the University Medical Center Utrecht. All parents gave written informed consent after

full explanation of the study. Post-processing included semi-automatic histogram intensity analyses to obtain estimates of cranium and total brain and total ventricular volume. Intrarater reliabilities were estimated using intraclass correlation coefficients and were > 0.98 for all measures (for more details, see Durston et al., 2004). After radiological evaluation, three children were found to have a left temporal cyst (which was not included in the total brain volume). One boy with mental retardation had a subcortical band heterotopia, which has no known implications for brain volume. All statistical analyses were repeated without these children.

Analyses were conducted using the SPSS statistical package (version 11.5). Bivariate and partial Pearson correlation coefficients were calculated. Significant correlations were followed up with linear regression analysis.

Results

Mean developmental quotient was 81 (SD=18, range 39-107). There was a strong correlation between intracranial and total brain volume ($r=0.96$; $p<0.01$). There were no significant correlations between intellectual functioning and intracranial volume ($r=0.10$; $p>0.1$) or between intellectual functioning and total brain volume ($r=0.30$; $p>0.1$). There was a significant correlation of 0.74 between total brain volume and intellectual functioning after partialling out intracranial volume ($p<0.01$). The model predicted by the linear regression analysis explained 56 % of the variance in intellectual functioning ($F=6.24$; $p=0.017$), see figure 1.

Significant negative correlations were found between intellectual functioning and total ventricular volume ($r=-0.77$; $p<0.01$). Findings were not significantly altered by partialling out age, gender, weight, height, or level of parental education, or after exclusion of the child with the SON-R or the children with radiological abnormalities. Findings did not alter when raw scores were used instead of age equivalent measures of the Mullen.

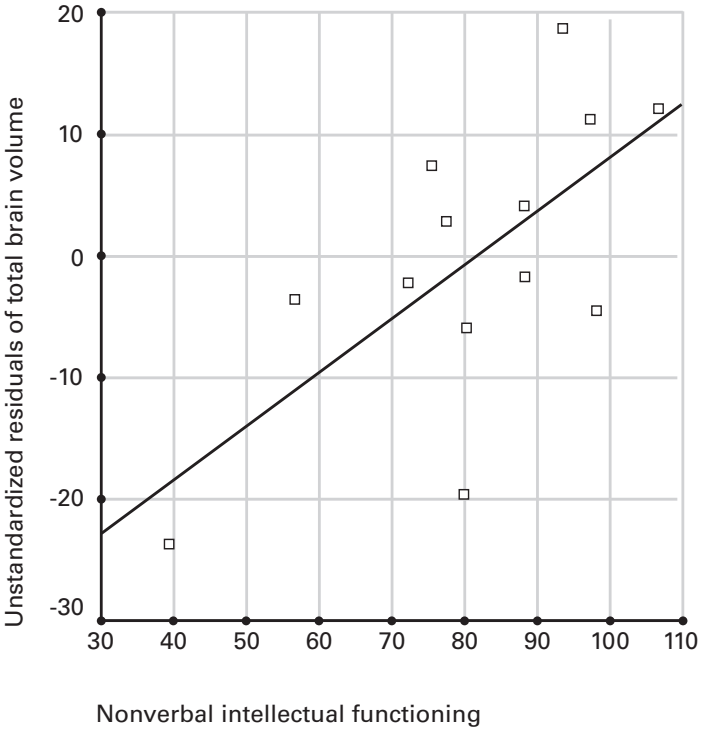

Figure 1 *The relationship between non-verbal intellectual functioning and the unstandardised residuals of total brain volume while controlling for intracranial volume in 13 children with a developmental delay below the age of five years.*

Discussion

The relationship between intellectual functioning and brain volume was investigated in children under five years of age. There was a strong correlation between brain volume and intellectual functioning after partialling out intracranial volume, where brain size relative to head size accounted for 56% of the variance in intellectual functioning at this age in developmentally delayed children.

Previous studies have reported associations between cerebral volume and intellectual functioning in (pre-)adolescent children (Wilke et al., 2003; Reiss et al., 1996). We did not find a relationship between intracranial or total brain volume with intellectual functioning by itself, but rather a relationship of brain volume with intellectual functioning when correcting for intracranial volume. This difference

may be related to our sample of developmentally delayed children.
In the first few years of life, intracranial volume increases partially under the influence of total brain volume growth (Dyke et al., 1933). Brain growth may or may not have been abnormal in early development, but the finding of a relationship between intellectual functioning and total brain volume only when controlling for intracranial volume suggests that there are later effects on total brain volume. This is in keeping with our finding of negative correlations between intellectual functioning and ventricular volume, which may be interpreted to indicate atrophy. As such, these results implicate the relevance of subtle regressive events in brain development in children with a developmental delay.
In this sample, three children were found to have intracranial cysts. Soto-Ares and colleagues (Soto-Ares et al., 2003) reviewed MR images of children with non-specific mental retardation. They reported cysts in 10% of their population, similar to the findings in this report. Our findings are based on a small sample of very young children with idiopathic developmental delay not related to a biological cause, as established by our extensive medical assessment. Taken together with the results from Soto-Ares and colleagues, this leads us to believe that, although our sample is small, it is representative of children with developmental delay.
Although we included a unique sample of very young children, with data on intellectual functioning and brain volumetric measures, this study was limited by small sample size. It took several years to acquire a group of young developmentally delayed children with no underlying biological substrate. As such, these results should be considered preliminary and need to be replicated in larger samples. Furthermore, as this sample was limited to children with subnormal to normal intellectual functioning, inferences regarding children with a higher level of intellectual functioning are precluded.

In sum, we report a correlation between total brain volume and intellectual functioning in a group of very young children with idiopathic developmental delay, when intracranial volume is controlled for, whereas there was no correlation between intellectual functioning and total brain volume or intracranial volume. These results suggest that there may be relative brain volume loss related to poor intellectual functioning in young children with idiopathic developmental delay.

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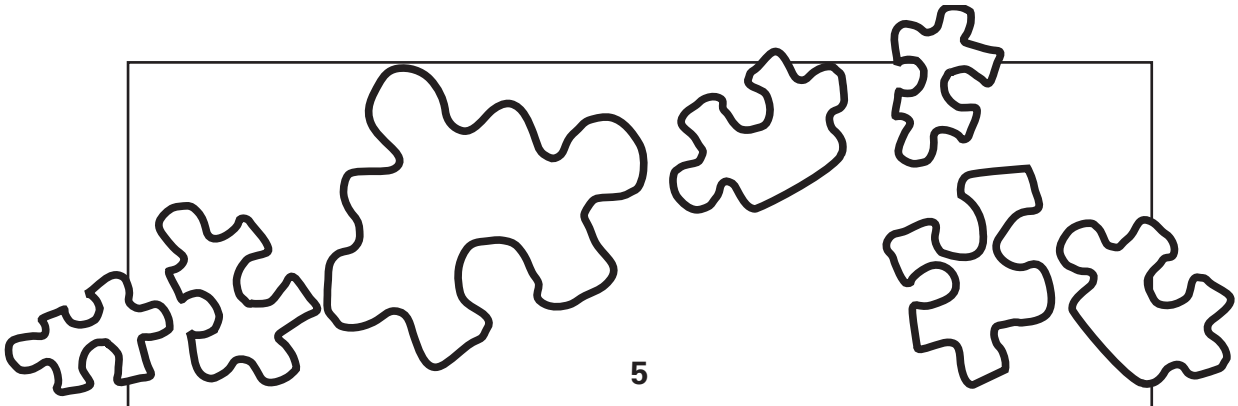

5

No differences in MR-based volumetry between 2-7 year old children with autism spectrum disorder and developmental delay

Mijke Zeegers, Hilleke Hulshoff Pol, Sarah Durston, Hilde Nederveen, Hugo Schnack, Emma van Daalen, Sophie Swinkels, Herman van Engeland, Jan Buitelaar

Abstract

Objective

To study brain volumes in children with ASD as compared to children with a mental retardation or a language delay (developmentally delayed). In addition, to study the association of intellectual functioning on brain volumes in children with ASD or developmental delay.

Methods

34 children with ASD and 13 developmentally delayed children without ASD, between two and seven years old, matched on age and developmental level, participated in a MRI study. Volumes of cranium, total brain, cerebellum, grey and white matter, ventricles, hippocampus and amygdala were measured.

Results

No significant differences in volumes of intracranium, total brain, ventricles, cerebellum, grey or white matter or amygdala and hippocampus between the ASD group and the developmentally delayed group were found. In the developmentally delayed group, a significant correlation (0.73) was found between intellectual functioning and total brain volume after partialling out intracranial volume. In the ASD group the correlation between intellectual functioning and brain volume corrected for intracranial volume was not significant.

Conclusion

No evidence was found for overall differences in brain volumes in children with ASD compared to developmentally delayed children between 2 and 7 years. The finding that higher intellectual functioning was not associated with a relative larger brain volume in children with ASD may suggest that a relative enlargement of the brain may not be beneficial to patients with autism.

Introduction

Autism is a pervasive developmental disorder characterised by a triad of social deficits, language and communication problems and a pattern of stereotyped, repetitive and restricted behaviours and interests (American Psychiatric Association, 1994). It is a clinically heterogeneous condition that has a broad range of severity and is frequently associated with concomitant learning disabilities. A wide variety of brain abnormalities has been reported by neuroanatomic and neuroimaging studies (Lainhart, 2006; Penn, 2006; Palmen et al., 2004; Palmen and van Engeland, 2004). Although the clinical manifestations of the disorder by definition are present before age three, brain development may be impacted much earlier, possibly as early as the first year of life (Courchesne et al., 2003). Three studies have been published that were specifically aimed at children under the age of five (Hazlett et al., 2005; Sparks et al., 2002; Courchesne et al., 2001) - see also table 1. The first study published (Courchesne et al., 2001) reports that by ages two to four years, 90% of autistic boys had a brain volume larger than normal average and 37% met criteria for developmental macrencephaly (brain volume that exceeds 2 SD above the normal mean). Autistic two- and three-year olds had more cerebral (18% volume increase) and cerebellar white matter (39% volume increase), and more cerebral cortical grey matter (12% volume increase) than normal. Sparks and colleagues (Sparks et al., 2002) also found significantly increased cerebral and cerebellar volumes in children with ASD compared with typically developing and developmentally delayed children. In the most recent study, significant enlargement was detected in cerebral cortical volumes but not in cerebellar volumes in individuals with autism (Hazlett et al., 2005). Enlargement was present in both grey and white matter, and it was found throughout the cerebral cortex. In the first two studies of children with autism between two and five years, brain volume was found to be approximately 10% larger than in typically developing children (Sparks et al., 2002; Courchesne et al. 2001). In the third study, in which the children were younger, this was approximately 5% (Hazlett et al., 2005).

We report here the results of our study of brain volumes of 47 children with an autism spectrum disorder, mental retardation, or a language delay between the

	Courchesne et al.	Sparks et al.	Hazlett et al.	Zeegers et al.
Participants with ASD (male/female)	30 autism (30m/0f)	45 ASD (38m/7f)	51 autism (46m/5f)	34 ASD (31m/3f)
Mean age (SD)	Not given	47.4 (4.2) months	2.7 (0.3) years	44.6 (11.3) months
Age range	2-5 years	38 - 54 months	18-35 months	21-77 months
Typically developing control children (male/female)	12 (12m/0f)	26 (18m/8f)	14 (10m/4f)	
Mean age in months (SD)	Not given	47.5 (6.2) months	2.4 (0.4) years	
Age range	2-5 years	36-56 m months	Not given	
Developmentally delayed control children (male/female)		14 (6m/8f)	11 (5m/6f)	13 (12m/1f)
Mean age in months (SD)		47.5 (5.6) months	2.7 (0.4) years	41.3 (11.8) months
Age range		40-58 months Matched to ASD on developmental level	Not given	26-62 months Matched to ASD on age and developmental level

Table 1 *Comparison of studies.*

ages of 21 to 77 months. Recently, we found an association between intellectual functioning and total brain volume after partialling out intracranial volume in the developmentally delayed group (Zeegers et al., submitted). Therefore, we also examined the correlation between intellectual functioning and brain volume in the ASD group.

Methods

Children were recruited from referrals to the Department of Child and Adolescent Psychiatry of University Medical Center Utrecht. Patients were included in the study if they were 18 months to seven years of age and were diagnosed with ASD, mental retardation, or language disorder. Children having significant motor or sensory impairment (e.g., blindness, deafness), major physical abnormalities, history of serious head injury, identifiable neurologic disorder (except epilepsy) or metal implants, such as prostheses, were excluded. Diagnosis was established according to DSM-IV criteria (American Psychiatric Association, 1994) and was based on all available information gathered since initial referral. This included a standardised behaviour observation, the Autism Diagnostic Observation Schedule (ADOS-G, Lord et al., 2000) and the standardised parent interview, the Autism Diagnostic Interview-Revised (ADI-R, Lord et al., 1994). The inter-rater reliability for the clinical diagnosis among three child psychiatrists (HE, JB, ED) was calculated for two diagnostic categories; ASD or other than ASD. Agreement corrected for chance was 0.74 (Cohen's kappa). Diagnostic discrepancies were resolved at a consensus meeting. Children younger than 42 months at the time of scanning, were re-evaluated when they were approximately 42 months old for a final diagnosis.

Forty-seven children participated in the MRI-study. The ASD group included 34 children (31m/3f) of whom 21 children had a diagnosis of autism and 13 were diagnosed with PDD-NOS (mean age 44.6 months, SD=11.3, range 21-77 months). The developmentally delayed group included 13 children (12 m/1f, mean age 41.3 months, SD=12, range 26-62 months), diagnosed with mental retardation (n=5, one child with borderline retardation, three with mild retardation and one child was moderately retarded) or with significant language delay (n=8). Groups were matched on age and developmental level.

All participants completed an extensive medical assessment (van Daalen et al., in preparation). A developmental paediatrician performed a physical examination and medical history, including assessment of perinatal circumstances and maternal illness during pregnancy. An audiologist and speech and language therapist evaluated hearing and language. All subjects were admitted to the neuropaediatric ward of the UMC Utrecht for an extensive blood screening, including amino acids, thyroid function, a karyogram, Fragile X testing and screening for metabolic disorders. The admission included consults by a paediatrician, child neurologist, and clinical geneticist. A lumbar puncture procedure was performed to assess the presence of metabolic abnormalities. Three children were born prematurely between thirty and thirty-four weeks of gestation with weights accordingly. No children were born with a birth weight below fifteen hundred gram. No metabolic disorders were found. All children had a karyogram as expected according to gender and none were positive on Fragile X. No children in this sample tested positive for the velo-cardio-facial syndrome deletion. Two children were treated with valproic acid for symptoms of epilepsy; one boy with symptoms of autism and absences, no mental retardation, and one boy with a subcortical band heterotopia, subsequent mental retardation and no autistic symptoms. Two children with autism used thioridazine for excessive fear and panic attacks in which they displayed severe self-destructive behaviour. Unfortunately, a control group of typically developing children could not be included as the children were below the age permitted for inclusion in MR studies for research purposes as stated by the Dutch Central Committee on Research involving Human Subjects.

The study protocol was approved by the Medical Ethical Review Board of the University Medical Center Utrecht. All parents of participating children with ASD or developmental delay gave written informed consent after full explanation of the study.

Intellectual functioning

Twenty children were administered the Mullen Scales Early Learning (Mullen, 1995) to measure developmental level. Several children however performed at floor of standardised scores. Therefore, it was decided to convert raw scores to a

developmental quotient in order to allow us to look more closely at the functioning of the more impaired children. Four subtests of the Mullen Scales were used; visual receptive, fine motor, receptive language, and expressive language – the fifth subtest, gross motor development, was not included. The developmental quotient was calculated as Mean Age Equivalent of the four subtests/Chronological Age x 100. For ten children (nine patients with autism and one patient with PDD-NOS), the Psychoeducational Profile – Revised (Schopler et al., 1990) was used to assess the developmental age. For six children, either the Griffith (Griffith, 1986) (one patient with autism, one with PDD-NOS and one child with language disorder), the Dutch Snijders-Oomen non-verbal intelligence test (Tellegen et al., 1996) (for one patient with autism) or the Kaufman Assessment Battery for Children (Kaufmann and Kaufmann, 1983) (for two patients with PDD-NOS) was used. Eleven children were not able to complete any of the offered tests, due to lack of cooperation or anxiety.

Brain imaging

The MR procedure was performed, while patients were under full anaesthesia with sevofluran. The following scans were acquired on a Philips NT Gyroscan scanner (Philips Medical Systems, Best, The Netherlands), operating at 1.5 Tesla; a T1-weighted three-dimensional fast field echo (FFE) scan with 130 to 150 1.5-mm contiguous coronal slices of the whole head (TE=4.6 ms, TR=30 ms, flip30°, FOV256 mm, in-plane voxel size 1 mm x 1 mm), and a T2-weighted dual echo turbo spin-echo scan with 65 to 75 3.0-mm contiguous coronal slices (TE1=14 ms, TE2=80 ms, TR6.350 ms, flip90°, FO256 mm, in-plane voxel size 1 mm x 1 mm). 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.

After radiological evaluation, four children were found to have arachnoid cysts, three were left temporal cysts; one was located near the cerebellum (cysts were not included in the total brain or cerebellar volumes). One male child was reported to have a subcortical band heterotopia. A girl with PDD-NOS was found to have a Chiari I malformation.

MR scans were coded to ensure masking for subject identity and diagnosis.

Analysis consisted of placing the MR scans in a Talairach frame without scaling (anterior commissure–posterior commissure alignment), followed by correction for inhomogeneities in the magnetic field (Sled et al., 1998). Intracranial volume, total brain volume, lateral ventricles, third ventricle, and cerebellum were measured automatically using histogram analysis algorithms and a series of mathematical morphology operators to connect voxels of interest within the cranium (Schnack et al., 2001a). Segmentations were then checked visually and edited manually, if necessary. Maps of cerebral grey and white matter were obtained using a histogram analysis algorithm (Schnack et al., 2001b). Hippocampal and amygdaloid volumes were traced manually by a single experienced rater (HN). 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 (Baare et al., 2001). Briefly, amygdala segmentation started in the first coronal slice where it was discernable, after the anterior commissure no longer appeared as a continuous tract. The lateral border was defined by the surrounding white matter and the inferior horn of the lateral ventricle. Posterior, the amygdala is bordered by the hippocampus. The medial border is formed by the cerebrospinal fluid, excluding the entorhinal cortex. Segmentation of the hippocampus started in the coronal slice in which the characteristic oval shape of mammillary bodies was visible for the first time and stopped when the fornix was visible as a continuous tract. Intrarater reliabilities were estimated using intraclass correlation coefficients and were 0.998 for both intracranial and total brain volume, 0.984 for cerebellar volume, 0.999 for lateral ventricle volume, 0.85 for third ventricle volume, and 0.83 and 0.84 for left and right amygdala, and 0.86 and 0.91 for left and right hippocampus.

Statistical analysis

Analyses were conducted using the SPSS statistical package (version 11.5). Analysis of variance was used to assess differences between groups in demographic variables. Differences in brain volumes were analysed by two-tailed general linear model (multiple) analysis of covariance. Although age and intracranial volume did not significantly differ between groups, we included both as a covariate in all analyses. To test if correlations of brain volumes and intellectual functioning are similar between the ASD and developmental delay groups, we repeated the statistical analyses of the previous study (Zeegers et al., submitted); i.e. unstandardised residuals of total brain volume after controlling for intracranial volume were correlated with IQ measures. Fisher's R to Z transformation was used to compare Pearson's correlation coefficients between groups. All statistical analyses were repeated without the children with radiological abnormalities.

Results

The ASD (34 children) and developmental delay group (13 children) were matched on age and developmental level (see also table 2). There were no group differences in gender, height, weight, or head circumference. No significant differences between the ASD group and the developmentally delayed group were found for any of the brain volumetric variables (intracranial volume F(1.44)=0.324, p=0.572; total brain volume F(1.44)=0.169, p=0.683; cerebral white matter F(1.44)=0.010,

	ASD	**DD**
N	34	13
Age in months, mean (SD)	44.6 (11.3)	41.3 (11.8)
Level of intellectual functioning, mean (SD)	64.2 (23)	73.6 (17.8)
Height in cm, mean (SD)	102.3 (7.7)	100.7 (10.9)
Weight in kg, mean (SD)	17.4 (3.2)	16.9 (3.5)
Education of parents (SD)*	12.0 (2.1)	13.2 (2.2)

* Highest number of years of education (any parent) after age 6

Table 2 *Sample characteristics.*

p=0.921; cerebral grey matter F(1.44)=0.386, p=0.538, total ventricular volume F(1.44)=0.254, p=0.617; cerebellar volume F(1.44=0.679, p=0.414, see also table 3). Amygdala and hippocampal volumes did not significantly differ between groups. Results did not change after exclusion of the female subjects, nor after exclusion of the patients with radiological abnormalities.

		IC	TB	Cerebral GM	Cerebral WM	Vent	CB	Amy	Hip
ASD	mean	1346.2	1252.7	739.5	368.2	9.0	134.8	2.5	6.0
	SD	134.6	125.6	66.0	57.1	7.4	14.0	0.6	0.8
DD	mean	1362.7	1262.5	755.0	366.1	10.0	131.0	2.4	6.4
	SD	124.8	122.3	65.4	64.8	6.2	15.0	0.7	0.8

Table 3 *Mean brain volumes, IC = intracranial volume, TB = total brain volume, cerebral GM = cerebral grey matter, cerebral WM = cerebral white matter, vent = total ventricular volume, CB = cerebellar volume, amy = left and right amygdala volume, hip = left and right hippocampal volume*

Correlation between intellectual functioning and brain volumes

Thirty-one children had a full-scale IQ score. No significant correlations were found between intellectual functioning and intracranial volume for either the ASD group or for the developmental delay group (r=0.09, p=0.71 and r=0.14; p=0.71, respectively) or between intellectual functioning and total brain volume (r=0.10, p=0.67 and r=0.32; p=0.37, respectively). For the developmentally delayed group there was a significant correlation of 0.73 between total brain volume and FSIQ after partialling out intracranial volume (p=0.02). For the ASD group the correlation was not significant at r=0.06 (p=0.81). Fisher's R to Z confirmed that the correlation coefficients differed significantly (z=1.98, p=0.02) (see figure 1).

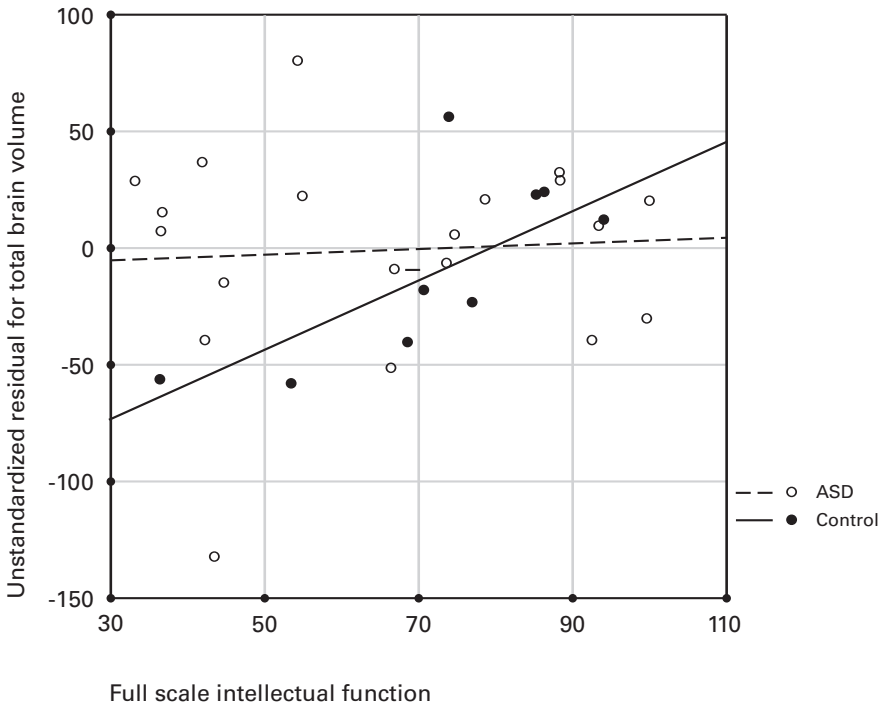

Figure 1 *The relationship between full scale intellectual functioning and the unstandardised residuals of total brain volume while controlling for intracranial volume (N=31).*

Discussion and conclusion

In this study, we report on brain volumes in young children with autism spectrum disorder (ASD) and in young children with developmental delay. We measured intracranial volume, total brain volume, cerebral grey and white matter volume, ventricular and cerebellar volumes, and manually traced amygdala and hippocampal volumes. Overall, there were no significant differences in brain volumes between the ASD and developmentally delayed children. A correlation of 0.74 between intellectual functioning and total brain volume - after partialling out intracranial volume - reported for the developmentally delayed group, was not found in the ASD group. All children were below the age of seven years (21 to 77 months old) at the time of scanning.

Previously, brain volume in children with autism was found to be approximately 5-10% larger than in typically developing children. The absence of larger brain volumes in our ASD participants may, to some extent, be attributed to populations studied. Possibly different subgroups were included in our study as compared to earlier studies (Hazlett et al., 2005; Sparks et al., 2002; Courchesne et al., 2001). We, for example, found comparatively more children with radiological abnormalities than did other studies, see Zeegers et al., in press. This might be related to possible differences in aetiology of ASD. However, the children with radiological abnormalities did not differ in brain volumes compared to those children without radiological abnormalities.

Studies of subjects with ASD are complicated by the confounding effects of co-existing mental retardation, which is associated with smaller head sizes (Sanchez Lastres et al., 2003; Watemberg et al., 2002; Jaffe et al., 1988). A recent study by our group (van Daalen et al., submitted) analysed head circumference data of 112 children with ASD or developmental delay (of which 33 children are included in this study). They found that mental retardation was associated with average head circumference at birth and subsequent head growth retardation, compared to overall body growth. ASD, on the other hand, was associated with average head size at birth and subsequent abnormal high head growth rate, compared to overall body growth. Head growth of infants with ASD in the first year of life can be expressed as a hyperbola function. At the end of the first year, the head growth restraining effect of mental retardation diminishes, whereas the head growth stimulating effect of ASD is still constant. For children with low functioning ASD, head size at a certain age is likely to be dependent on the additive effects of mental retardation and ASD. When assessing head circumference of the children participating in this study at the time of MR scanning, we found no differences between the groups ($F(1.44)=0.268$; $p=0.608$). Head circumference did show a high correlation with intracranial volume (0.86 for the ASD group and 0.69 for the developmentally delayed group). This suggests two possibilities. First, a selection bias could have led to the inclusion in this MR study of a subgroup of children not representative of the children from the van Daalen et al. study. Or, alternatively, early abnormalities of growth of head size in relation to body length as documented in the first year of life, have disappeared when examined by volumetric MRI in later

years. The differential effects of ASD and mental retardation on the development of brain volumes over age remains to be further investigated.
Finally, and importantly, previous studies included a group of typically developing children for comparison. Unfortunately, we could not include a control group of typically developing children, as these would have been below the age permitted in MR studies. With our developmentally delayed group also severely affected, it was possible for us to match the ASD group to the developmentally delayed children on both developmental level and chronological age. Only the Sparks study (Sparks et al., 2002) matched the ASD and developmental delay groups on developmental level. Possibly, differences in results may be explained by matching differences.

Correlation between intellectual functioning and brain volumes

In the developmentally delayed group, there was a strong correlation of 0.73 between brain volume and intellectual functioning after partialling out intracranial volume (Zeegers et al., submitted). Brain size relative to head size accounted for 56% of the variance in intellectual functioning in these children, suggesting that there may be relative brain volume loss related to poor intellectual functioning in young children with idiopathic developmental delay (Zeegers et al., submitted). In the current study, however, the ASD group showed no significant correlation (0.06).
In a previous study of 67 subjects with high functioning autism between eight and 46 years, no significant correlation between brain volumes and total IQ, performance IQ or verbal IQ was reported (Aylward et al., 2002). A positive correlation was found in 21 patients with Asperger syndrome, but in that same study a negative correlation for high functioning autism and no correlation for the low functioning patients was found (all patients were between the ages of seven and 18) (Lotspeich et al., 2004). In our study, increased brain size in autism appears to disrupt at least one typical brain size correlate; the positive correlation of brain size with intellectual functioning (e.g. Reiss et al., 1996; Andreasen et al., 1993). In autism, there seems to be a lower degree of coordination and communication between cortical areas than in normal controls (Just et al., 2004). Co-occurrence of reduced functional connectivity and reduced white matter (e.g. Courchesne et al.,

2001; Berthier et al., 1993) makes some sense as a possible explanation, given the physical constraints on how large the brain can grow, while still maintaining adequate levels of connectivity (Ringo, 1991). The fact that no association between intellectual functioning and brain volume was found, suggests that extra tissue is not well-integrated functionally in autistic subjects, whereas it is in developmentally delayed children. Therefore, a relative enlargement of the brain may not be beneficial to patients with autism.
A recent longitudinal study showed that the level of intelligence of typically developing children was related to the pattern of cortical growth in childhood and adolescence, indicating that the neuroanatomical expression of intelligence in children is dynamic (Shaw et al., 2006). This might indicate that the absence of a correlation between intellectual functioning and brain volume could be momentary and that, when measured earlier or later, a significant relationship might occur. More longitudinal studies are needed to clarify this issue.
Although we included a unique sample of very young children, with data on intellectual functioning and brain volumetric measures, this study was limited by a small sample size. As such, these results should be considered preliminary and need to be replicated in larger samples.

In conclusion, we found no significant differences in brain volumes between the ASD and developmentally delayed children. In contrast to a significant correlation that was previously reported for the young developmentally delayed children (Zeegers et al., submitted), we found no significant correlation between total brain volume and intellectual functioning, when corrected for intracranial volume, in the children with ASD. These results suggest that a relative enlargement of the brain may not be beneficial to patients with autism.

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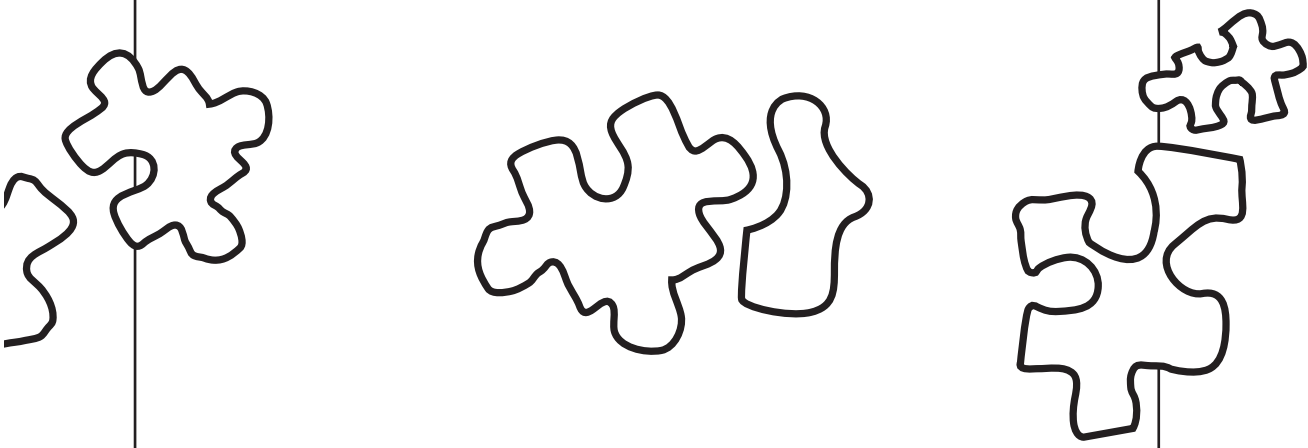
General discussion and conclusions



This thesis concentrates on brain development in young children with ASD, examining brain volumes and brain metabolism. The main aims of this thesis are to study the MR morphologic and spectroscopic brain correlates in children with an atypical development that came to the attention of the Department of Child and Adolescent Psychiatry before the age of three years. We investigate whether, at a very early age, children with ASD differ from children with a developmental delay without ASD in metabolic, radiologic, or volumetric aspects of their brain development.

Strengths of the studies

When the studies leading to this thesis commenced, there had been no studies of MRI or MR spectroscopy in children with autism between the ages of two and seven years. A few studies concerned with head circumference had been published (Lainhart et al., 1997; Davidovitch et al., 1996; Woodhouse et al., 1996). The estimate was that about one-third of children with PDD had macrocephaly (Woodhouse et al., 1996) - the current estimate is around 19 % (Dementieva et al., 2005). The timing of the onset of head enlargement, now set in the first year of life (Dementieva et al., 2005; Courchesne et al., 2003), was unknown.

The studies included in this thesis were aimed at describing the autistic brain at a very young age. A major strength of this study is the inclusion of 48 toddlers with

ASD or developmental delay with the youngest child in the study group being 21 months of age at the time of scanning. Another strength of these studies is the large amount of other data available on the majority of included children, those who were part of the SOSO project (see also below). The SOSO project collected questionnaires, developmental and medical histories, observations, test results of intellectual functioning, attachment qualities, language levels, autism rating scales and much more. For the studies in this thesis, this wealth of information meant that the children were well described.
Autism research is complicated by the choice of control groups. Especially when low-functioning participants are included, it is important to have a low-functioning comparison group. We were able to include a low-functioning group of children with mental retardation and a group of children with language disorder, enabling us to match the ASD group to the control group for level of intellectual functioning. Matching for intellectual functioning ensures that differences between groups are not caused by differences in intellectual functioning.

The SOSO project

The aim of this thesis was to study the brain – behaviour relationships in children with ASD who were identified in a population-based screening. In two and a half years, 31.724 children aged 14 to 15 months were screened using the ESAT (Dietz et al., in press). Of the 73 infants eventually examined at the toddler unit of the University Medical Center Utrecht, 18 were diagnosed as having ASD at the first psychiatric evaluation. Other diagnoses included non-ASD developmental disorders, such as developmental language disorder (n=18), or general mental retardation (n=13). Another 11 children had problems that would fit other diagnostic categories of the DSM-IV. The remaining 13 children had problems that were best labeled by a Diagnostic Classification in the DC 0-3 system (National Center for Infants, Toddlers and Families, 1994). Parents of children diagnosed with ASD, language disorder or mental retardation were approached to participate in an extensive medical assessment (van Daalen et al., in preparation), including MRI and – for research purposes – MR spectroscopy. Unfortunately, many parents of children with language disorder or mental retardation did not consent to participating in

the medical assessment for various reasons. Furthermore, parents of children with ASD often did not comply or only consented to blood and urine testing. This was in part due to the length of the protocol (children were admitted to the hospital for two days) for parents already burdened with a child with developmental problems. After diagnosis had been established, parents had been offered care as usual and many stated that life was finally settling down again, their child was showing some improvements in behaviour, and they were not willing to risk a disruption from normal life, such as hospital admittance.

Finally, 34 children included in the SOSO project also participated in the studies described in this thesis. Of these children, 16 were identified by the ESAT screening and 18 were included later, as physicians at the well baby clinics were asked to also refer older children with suspected ASD. Of these 34 children, 12 were diagnosed with autism, nine with PDD-NOS, five with mental retardation, and eight with language delay. To increase the number of participants, the medical assessment was also offered to nine children at the toddler unit of the Department of Child and Adolescent Psychiatry of University Medical Center Utrecht (six children with autism, two with PDD-NOS and one with language disorder) and to five children from the Radboud University Medical Center Nijmegen (three with autism and two with PDD-NOS). Despite substantial efforts to include additional subjects with possible ASD, mental retardation, or language disorder, it was difficult to increase the number of subjects in these studies.
The aim of studying a pure population-based sample with ASD turned out not to be feasible. On the other hand, including these population-screened children in our study may have lead to a different group of participants compared to previous studies of young children with ASD. Children identified by population-based screening may be different in terms of behaviour, development and perhaps even biology compared to children referred after expression of parental concerns.

Are autism spectrum disorders at a very young age associated with radiological abnormalities of the brain?

In chapter 2, architectonic brain development is examined. Brain development is an intricate process during which many things can go wrong. We examined the MR images of the participating children for the prevalence of brain abnormalities, such as cortical migration disorders, ventricular dilations, and disturbed myelination patterns. In almost half of the population, abnormalities were reported. These ranged from (harmless) corpus callosum shape variations to a myelination delay of 11 months in a 21-month old girl. One of the children was found to have a Chiari I malformation and four children were diagnosed with an arachnoid cyst. In a report on the occurrence of radiological abnormalities in a healthy paediatric population, incidental abnormalities were detected in 12 % of the subjects - not including sinusitis (Kim et al., 2002). Our 49% occurrence of radiological abnormalities is in sharp contrast. There have been discussions about the clinical relevance of MR scanning in children with a developmental delay or autism. The current view on imaging of children with autism is that the yield of MRI is low, as most findings are viewed to be incidental (Kosinovsky et al., 2005). Identified in this study were a Chiari I malformation and several arachnoid cysts, which can give rise to severe headaches. Children with a developmental delay (with or without autism) are often not as able as typically developing children to communicate pain. The identification of possible pain inducing brain abnormalities may alert the physician and parents to physical discomfort that perhaps cannot be fully expressed by the child. Therefore, we believe that in some cases imaging may be relevant to clinical practice.

It is unlikely that brain imaging will aid the diagnosis of ASD. Nor is autism associated with specific brain abnormalities. However, in this study we reported a high percentage of radiological abnormalities in very young children with an atypical development. Our report of 49% of cases is substantially higher than has been reported in a healthy paediatric population and in populations of older children, adolescents, or adults with ASD. Children diagnosed with ASD at a very young age may differ from children who are not seen until school age or pre-adolescence, who are more typically included in MR studies. The first group of

children may show behaviour that is more deviant, and have a lower level of functioning than the latter group (Lord, 1995). Therefore, children who are identified with developmental disorders at an early age may also differ in the amount or severity of brain abnormalities compared to children that were identified at a later age. We believe that the children with ASD or developmental delay who are most likely to benefit from MR imaging are those that present with misunderstood and unmanageable behaviour, which might be caused by pain that cannot fully be expressed by the child.

Are autism spectrum disorders at a very young age associated with decreased neuronal integrity?

Chapter 3 concentrates on measurements made using proton magnetic resonance spectroscopy. This MR tool is not often used in child psychiatry research. It was included in this study to compare brain metabolism in boys with ASD to boys without ASD. We hypothesised, based on previous literature, a decreased neuronal integrity in autism - as reflected in a lower level of N-acetyl-aspartate (NAA). The measurements were taken in the frontal subcortical white matter and the amygdala-hippocampal area, areas indicated to be involved in autism (Araghi-Niknam and Fatemi, 2003; Kemper and Bauman, 1998), in the left hemisphere, which is thought to be more dysfunctional than the right (Chiron et al., 1995; Dawson, 1983). One of the important issues in spectroscopy in young children is the ongoing development of metabolic concentrations. Developmental studies have shown that the concentration of NAA, creatine, and glutamine and glutamate increases with brain development, whereas the concentration of choline and myo-inositol decreases with brain development (Friedman et al., 2003; Bhakoo and Pearce, 2000; Moore, 1998; Kreis et al., 1993). Even though the most rapid changes have been noted during the first three years of life, changes have still been observed up to the age of 16 years (van der Knaap et al., 1990). In this respect, we believe it is important to keep the age range of both patient and (age matched) control group as small as possible. Even then, including age as an independent covariate in data analysis seems obligatory. Spectroscopic analyses also included intellectual functioning as a covariate, since stage of development has an effect on metabolite levels as well

(Filippi et al., 2002). When comparing a group of (retarded) autistic children with a group of non-autistic children with a different level of intellectual functioning, autism itself as well as mental retardation may underlie metabolic differences. In accordance with studies of typically developing children (Friedman et al., 2003; Bhakoo and Pearce, 2000; Moore, 1998; Kreis et al., 1993), we found a positive correlation between increasing age and levels of creatine and NAA in the frontal subcortical white matter. Increasing NAA concentrations may reflect the process of differentiation and maturation of dendrites, axons, and synapses together with neuronal soma (Martin et al., 2001). As cerebral maturation proceeds, NAA levels rise. A rise in creatine is thought to be associated with myelination of the developing brain (Moore, 1998).

However, no differences were found between children with ASD and children with developmental delay in metabolite concentrations of NAA, choline, or creatine. Possible explanations of these results include the issue of localisation. Difficulty in localisation results in part from an absence of formal agreements about the cortical landmarks to be used in human MRS studies and the inability to establish isotropic cortical volumes in study subjects (Yurgelun-Todd and Renshaw, 2000). We measured metabolite concentrations in the frontal subcortical white matter and in the amygdala-hippocampus complex. Previous studies have measured metabolite levels in the parietal and occipital lobe (Filippi et al., 2002), the cerebellar hemispheres (Mori et al., 2001), the brain stem and cingulate gyrus (Hisaoka et al., 2001). As different brain regions have different levels of metabolic functioning, they cannot be easily compared to each other. Even though neuropathological and neuropsychological studies point to the white matter in the frontal lobe and to the amygdala-hippocampus, metabolite concentrations might not differ between ASD and controls. Still, decreased levels of NAA have been reported in the amygdala of (pre)pubertal children with autism (Mori et al., 2001) and spectroscopic imaging found lower levels of NAA in children with ASD in the frontal white matter (Friedman et al., 2003).

Secondly, technical difficulties precluded us from including all children in the final analysis. Even though a power analysis showed that over 500 children needed to be included in each group for the NAA levels in the frontal voxel to show a significant difference (see chapter 3), the analyses in the amygdala-hippocampal voxel could have benefited from larger control groups. The technique used to measure metabolite concentrations in this study allows manipulation of the voxel size to position the volume of interest away from non-relevant tissue. It proved difficult to exclude CSF and blood vessels from the amygdala-hippocampal voxel, while retaining a large enough volume for the measurement. The N in the mental retardation group was limited to two. Therefore, conclusions about the metabolite levels in the amygdala-hippocampal area are to be considered preliminary and results need to be replicated in larger samples.
Thirdly, a study needs meaningful control groups. We included two control groups, children with mental retardation and children with language disorder. The children with mental retardation were matched to the children with ASD for intellectual functioning. With the absence of a group of typically developing children, language disordered children were thought to be as close to typically developing as possible (as the measured brain areas are not relevant for language processing). Unfortunately, without a typically developing control group, no conclusions on deviations from typically developing individuals are possible. Based on this study, we cannot exclude the possibility of aberrant levels of metabolite concentrations in children with ASD, as well as in children with mental retardation and language disorder.

With the continuing development of metabolic concentrations in young children, with the large age ranges in most studies, and without controlling for intellectual functioning, previously reported differences in metabolite concentrations might be the result of differences between the groups in age and developmental level. Therefore, we strongly recommend controlling for age and intellectual functioning in MRS studies of young children.

Is there a relationship between brain volumes and intelligence in children with developmental delay at very young age?

In chapters 4 and 5, we turned to the volumetric MRI data. In chapter 4, we studied the relationship between intellectual functioning and brain volume. This chapter specifically included the children with mental retardation or language disorder without ASD. We chose to first examine the brain – intellectual functioning relationship in developmentally delayed children without ASD, as contradictory results are reported in studies including patients with ASD. In previous studies of typically developing children, brain volume has been shown to be associated with intellectual functioning; in (pre-)adolescent children correlations range between 0.20 and 0.28 (Wilke et al., 2003; Reiss et al., 1996). A very recent study showed that the level of intelligence of typically developing children was related to the pattern of cortical growth in childhood and adolescence, indicating that the neuroanatomical expression of intelligence in children is dynamic (Shaw et al., 2006). However, it remains unclear whether cognitive functioning and brain volume are associated in children under the age of five. Furthermore, little is known about the relationship between brain volumes and intellectual functioning in children with developmental delay. In this chapter, we aimed at identifying the relationship between brain volumes and intellectual functioning in low functioning children between two and five years of age. We found no significant correlation with intellectual functioning and intracranial volume or total brain volume. However, there was a correlation of 0.74 between intellectual functioning and total brain volume after correction for intracranial volume, meaning that brain size relative to head size accounted for 56% of the variance in intellectual functioning at this age in developmentally delayed children. Even though there has not been much research on the subject, intracranial volume is thought to increase under the influence of total brain volume growth (Dyke et al., 1933) - though some influence of genes or other processes seems likely (Liu et al., 1995). In our population of developmentally delayed children, brain growth may or may not have been abnormal in early development. The finding of a relationship between intellectual functioning and total brain volume only when controlling for intracranial volume, suggests (additional) later effects on total brain volume. Later growth may have

been stunted or developmental volume loss may have been accelerated. This is in keeping with our finding of negative correlations between intellectual functioning and ventricular volume, which may be interpreted to indicate atrophy. This may indicate that (lack of) brain growth during infancy and early childhood is important in determining cognitive functioning, and as such may indicate subtle regressive events in brain development in these developmentally delayed children.

Are autism spectrum disorders at a very young age associated with differences in brain volumes?

In chapter 5, brain volumes in the ASD group are compared to the volumes in the developmentally delayed group, examining whether brain volumes are increased in the children with ASD. Contrary to our hypotheses, we found no increase in the volume of cranium, total brain, ventricles, cerebellum, grey or white matter, amygdala or hippocampus in the ASD group. Three previous studies of autism in very young children reported a 5 - 10 % increase in brain volume in children with ASD compared to either developmentally delayed children or typically developing children (Hazlett et al., 2005; Sparks et al., 2002; Courchesne et al., 2001).

The absence of larger brain volumes in our ASD participants may be related to differences between studies, such as different subgroups of ASD patients, with different levels of functioning and presence or absence of epilepsy. Due to our severely affected developmentally delayed control group, it was possible for us to match the ASD children to the developmentally delayed children for both developmental level and chronological age. Previously, only the Sparks study (Sparks et al., 2002) had matched the ASD and developmentally delayed groups for developmental level. Possibly, differences in results may be explained by differences in control group matching strategy.

We could not replicate previous studies. Of course, previous studies included a comparison group of typically developing children. Unfortunately, we were not able to include a control group of typically developing children, as these would have been below the permitted age for inclusion in MR studies in the Netherlands. Our results might also be related to possible differences in aetiology of ASD. Indeed, the children that were included in our study had relatively more radiolog-

ical abnormalities than children included in the other studies (see also chapter 2), pointing to a possible different aetiology with a more severely affected brain.
In reference to chapter 4, the correlation of intellectual functioning with brain volume was re-examined in the ASD group. The positive correlation between intellectual functioning and total brain volume after controlling for intracranial volume in the developmentally delayed group, was not present in the ASD group. Normally, a correlation would suggest that extra tissue is beneficiary for intellectual functioning. The absence of a significant correlation between intellectual functioning and brain volume suggests that in autistic subjects extra tissue is not well-integrated functionally, whereas it is in developmentally delayed children. Therefore, a relative enlargement of the brain may not be beneficial for patients with autism. Another possibility is that brain function in autistic patients is different from brain function in patients with mental retardation. There may be different neurobiological processes underlying mental retardation in patients with autism compared to patients with mental retardation.

Summary and methodological issues

Overall, it proved difficult to replicate previously reported results. Using spectroscopy, we found no differences between the ASD and developmentally delayed groups. We believe that this may be related to the small age range of our participants and our stringent covariation for age and developmental level. Structural MRI found no volumetric differences between the two groups. This may be related to differences between in our patient and control groups compared to other studies.
Most volumetric studies to date have reported possible brain abnormalities as an aside. Therefore, they may miss more subtle radiological abnormalities, as they did not investigate them specifically. In chapter 2, we specifically aimed at identifying these abnormalities. This is the first study of radiological abnormalities in autism and developmental delay since 1990 (Piven et al., 1990).
The relationship between intellectual functioning and brain volume had never been described in children with a developmental delay under the age of five. We found a positive correlation between intellectual functioning and total brain

volume after controlling for intracranial volume, indicating that the growth of the brain in relationship to the cranium might differ in developmentally delayed from typically developing children. The absence of this relationship in children with autism is not new. However, it had not been previously studied in young children in whom both brain and cognition are still very much developing. We were able to extend the finding of an absent correlation between brain volume and intellectual functioning in autism to very young children.

In general, our findings deviate from the findings previously described in the literature. This applies particularly to the chapters on spectroscopy and volumetric imaging in this thesis. There are several methodological issues.
First, as the spectrum of ASD is wide, it is more than likely that there are differences amongst studies in the patients included. In recent years, research groups have tried to standardise inclusion. Groups are homogenised by complementing clinical diagnoses with ADI and ADOS diagnoses. This facilitates comparison across studies. However, this is difficult in the case of very young children. Their clinical diagnosis within the autistic spectrum is still unstable, particularly for children with atypical autism or PDD-NOS (Charman and Baird, 2002). Furthermore, some of the DSM-IV criteria are not applicable at this age (Volkmar et al., 2005) and also the ADOS and ADI are not particularly suitable for young children (Cox et al., 1999; Stone et al., 1999; Lord, 1995). The age range of the children, participating in the studies included in this thesis, covers a period of continuing development in intellectual functioning (Dietz et al., in press), and one of constant evolvement of symptoms, complicating comparison of children across even a small age range. Still, diagnostic reliability in this study was 0.74 (van Daalen et al., in preparation). Second, as MR imaging requires the ability to cooperate and lie motionless for prolonged periods of time, studies typically include high functioning children with ASD, as anaesthesia is usually only applied in the case of a very young, severely affected child undergoing an MR-scan for clinical purposes. Since ASD is difficult to identify in very young children with about or above average cognitive skills, studies that include young children are often limited to low functioning participants. This limits comparison across age groups and generalisability of results of older children to younger age groups and vice versa.

A third possible confound is the co-occurrence of mental retardation in ASD. Syndromes with mental retardation are generally associated with smaller head circumferences and brain volumes. Autism, on the other hand, is associated with larger head size and brain volumes. Analyses of head size data of very young children with ASD with and without mental retardation and very young children with mental retardation without ASD suggest that there are differential effects of ASD and mental retardation on head size (van Daalen et al., submitted). Therefore, the choice of a control group is very important to the interpretation of results. The studies described in this thesis include control groups of children with mental retardation and language disorder. It was not possible to include a control group of typically developing children as they were below the age permitted to be included in MR studies for research purposes, as stated by the Dutch Central Committee on Research involving Human Subjects. Ideally, one would include the three groups to allow for better comparisons.

Fourth, the studies described in this thesis included 48 children with autism, PDD-NOS, mental retardation, or language disorder. As mentioned before, despite substantial efforts to include additional subjects with mental retardation or language disorder, it was difficult to increase the number of subjects in this group. Difficulties identifying and scanning these children limited our ability to include a larger comparison group. Therefore, the power of analyses may have been curtailed.

A final issue concerns the measurements. Is it possible to measure brain volumes and metabolites accurately and consistently in young children? Volumetric imaging has a comparable methodology across different research groups. Scan sequences and post processing may differ slightly, but, in general, results are considered reliable and trustworthy. It is a different story for MRS. The field of autism spectroscopy suffers from a lack of replication across studies and poor methodology in terms of not controlling for confounding variables (Goldberg et al., 1999). Methods differ considerably across scanners and research groups, making interpretation and comparison of data difficult. With localised spectroscopy, even though it has demonstrated reliable quantitation (Stanley, 2002), the results are always dependent on the VOIs chosen and data appear to change with brain

location and age of the subject (Sokol et al., 2002). Results from spectroscopic imaging on the other hand cannot be directly compared to VOI measured concentrations, making comparison across studies even more difficult. However, the main problem is a lack of knowledge. It is unknown what the biological variation in metabolite concentrations is in typically developing children at any given age, let alone in children with ASD. With no information on the variation of concentrations, the N in a study may be too small to reliably accept or refute the null hypothesis. Including different ages in one group may further complicate matters.

Recommendations for further research

Can imaging be used as a research tool for studies of brain development in young children with an atypical development? Despite the limitations associated with various populations, the choice of control groups, and the large differences in age groups, we believe imaging of brain development in young children with ASD can be valuable. Imaging may provide an indication for the precise timing of brain maldevelopment, it's localisation, and the possible neurobiological mechanisms underlying it. However, it is important to keep patient groups as homogenous as possible. Preferably, included subjects would span a very small age range and have the same level of intellectual functioning. As with all developmental imaging, longitudinal studies are the method of choice for autism research. Further homogeneity may be accomplished by re-evaluating the children at a second time point to account for subsequent development of symptoms and intellectual functioning. Genetic-sensitive designs, such as high-risk studies of siblings of ASD patients, may be especially informative as they have the potential to decrease the heterogeneity in the phenotype.

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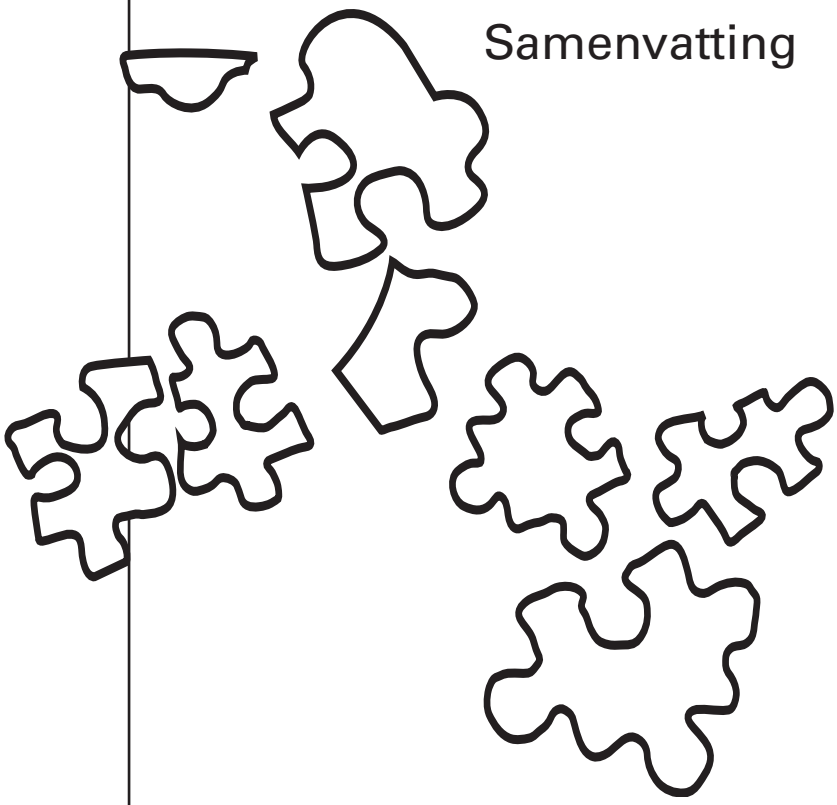
Samenvatting



De verzameling pervasieve ontwikkelingsstoornissen van het autistisch spectrum (ASD) omvat autisme, PDD-NOS (Pervasieve ontwikkelingsstoornis - niet anders omschreven) en het syndroom van Asperger (American Psychiatric Association, 1994). Een diagnose binnen het spectrum wordt gesteld op basis van problemen met sociale interactie, verbale en non-verbale communicatie en wanneer er beperkte en stereotypische interesses zijn, waarbij er iets meer nadruk ligt op het sociale aspect. Omdat de grenzen van het spectrum moeilijk vast te stellen zijn, is het ook moeilijk om te stellen hoe vaak ASD voor komt. De huidige schattingen komen uit op ongeveer 30 tot 60 patiënten per 10.000. Ongeveer een kwart van hen voldoet aan de criteria voor autisme, en iets minder dan de helft heeft een laag niveau van functioneren, een non-verbaal IQ van minder dan 70 (Chakrabarti and Fombonne, 2001). Autisme komt vaker voor bij jongens dan bij meisjes, ongeveer een verhouding van 3.5 of 4.0 : 1 (Fombonne, 2003; Lord et al., 1982). Er is een sterk effect van mentale retardatie (een IQ onder de 70) op deze incidentie; onder de hoogfunctionerende mensen met autisme bevinden zich meer jongens dan meisjes, terwijl de verhouding laag is bij laagfunctionerende mensen met ASD (Lord et al., 1982).

Autisme is voor een groot deel genetisch bepaald. Uit tweelingstudies blijkt dat er een 60-90% concordantie is voor eeneiige tweelingen, terwijl het 0-20% is voor twee-eiige tweelingen (Bailey et al., 1995; Steffenburg et al., 1989; Ritvo et al., 1985). De frequentie van ASD in broertjes en zusjes wordt geschat op 50 tot 150 (Folstein and Piven, 1991; Jorde et al., 1991; Smalley et al., 1988). Op basis van epidemiolo-

gisch onderzoek en onderzoek bij families en tweelingen gaat men ervan uit dat autisme een complexe genetische aanleg heeft, waarbij misschien 3 tot 4 genen een rol spelen, maar waarschijnlijker eerder 10 loci of meer (Risch et al., 1999; Pickles et al., 1995).
Voor schoolgaande kinderen en ouder is de diagnose van ASD erg stabiel; tussen de 72 en 87 % van de kinderen behoudt de diagnose na een herbeoordeling (Klin et al., 2004). Maar de diagnose van jonge kinderen is moeilijker. Het is inmiddels duidelijk dat een betrouwbare diagnose mogelijk is vanaf twee jaar (Moore and Goodson, 2003; Lord, 1995), echter kinderen met een vroege diagnose van atypisch autisme of PDD-NOS kunnen soms nog binnen het spectrum veranderen van categorie (Charman and Baird, 2002; Cox et al., 1999; Stone et al., 1999).

De onderzoeken die beschreven zijn in dit proefschrift waren een onderdeel van het SOSO project (ScreeningsOnderzoek Sociale Ontwikkeling). Doel van het SOSO was om kinderen met ASD al op de leeftijd van 14 maanden te identificeren. Daarna werd de kinderen die ASD of een andere ontwikkelingsstoornis leken te hebben gevraagd om deel te nemen aan een uitgebreide medische check-up, waar magnetische resonantie beeldvorming (MRI) en magnetische resonantie spectroscopie (MRS) deel van uit maakten. De resultaten van deze twee onderzoekstechnieken zijn beschreven in dit proefschrift.

Het doel van dit proefschrift was het bestuderen van de metabolische, radiologische en volumetrische aspecten van hersenontwikkeling van jonge kinderen met ontwikkelingsstoornissen; ASD, mentale retardatie of taalstoornis. Bij het begin van dit project waren er nog geen studies gepubliceerd die specifiek onderzoek deden naar deze leeftijdscategorie. We wilden de relatie tussen brein en gedrag bestuderen in deze jonge kinderen met ASD.
In hoofdstuk 2 zijn de resultaten beschreven van de beoordeling van de MR beelden door de radiologen. Zij hebben de scans onderzocht op de aanwezigheid van onder andere corticale migratiestoornissen, vergrotingen van de ventrikels en afwijkingen in myeline ontwikkeling. Bijna de helft van de onderzochte kinderen liet een afwijking van normaal zien, verschillend van een relatief onschuldige vormafwijking van het corpus callosum tot een myeline ontwikkelingsachterstand

van 11 maanden in een meisje van 21 maanden oud. Een van de kinderen bleek een Chiari I malformatie te hebben en vier kinderen hadden een cyste. Zowel cystes and Chairi I malformaties kunnen heftige hoofdpijnen veroorzaken. Kinderen met een afwijkende ontwikkeling, met of zonder autisme, hebben soms moeite met het communiceren van pijn. Het vinden van een mogelijk pijninducerende hersenafwijking kan ouders en behandelaars wijzen op de mogelijkheid van pijn in een kind dat dit zelf niet goed kan communiceren. Hoewel de huidige mening over imaging van jonge kinderen met autisme is, dat de opbrengst laag is en dat MRI niet bijdraagt aan de diagnose, zijn wij daarom van mening dat MRI in sommige kinderen wel degelijk bij kan dragen aan de klinische behandeling. Dit geldt met name voor kinderen met onbegrepen en onhandelbaar gedrag, dat veroorzaakt zou kunnen worden door pijn die niet door het kind kan worden geuit.

In hoofdstuk 3 wordt met behulp van MRS gekeken naar de neuronale integriteit van de frontale subcorticale witte stof en van het amygala-hippocampus complex. Op basis van de literatuur veronderstelden we een verlaagde neuronale integriteit, gemeten als een verlaagd niveau van N-acetyl-aspartaat (NAA), te vinden in hersenen van de kinderen met ASD, maar niet in de hersenen van de kinderen met andere ontwikkelingsstoornissen. Ontwikkelingsstudies hebben aangetoond dat de concentratie van NAA, creatine, glutamaat en glutamine verhoogt tijdens hersenontwikkeling, terwijl de concentratie van choline en myo-inositol lager wordt (Friedman et al., 2003; Bhakoo and Pearce, 2000; Moore, 1998; Kreis et al., 1993). Gedurende de eerste 3 levensjaren zijn deze veranderingen het grootst, maar veranderingen in concentraties door ontwikkeling zijn ook nog gemeten in kinderen van 16 jaar oud (van der Knaap et al., 1990). In aansluiting op deze studies vonden wij een positieve correlatie tussen leeftijd en concentraties van creatine en NAA in de frontale subcorticale witte stof. Verhoogde NAA concentraties wijzen op differentiatie en rijping van dendrieten, axonen en synapsen (Martin et al., 2001). Door de ontwikkeling van de hersenen, stijgt de concentratie van NAA. De verhoging in creatine niveaus tijdens ontwikkeling wordt toegeschreven aan het proces van myelinisering van het brein (Moore, 1998).
Er werden geen verschillen gevonden in metabolieten concentraties tussen de groep met ASD en de groep met andere ontwikkelingsstoornissen. Het is echter

niet mogelijk om definitieve conclusies over de metabolietenconcentraties in deze groepen te trekken op basis van dit onderzoek. Dit komt door de kleine groepen patiënten en door de afwezigheid van een controlegroep van normaal ontwikkelende kinderen. Kinderen van deze leeftijd kunnen niet geïncludeerd worden in een dergelijk onderzoek, zoals gesteld wordt door de Centrale Commissie Mensgebonden Onderzoek (CCMO).

Hoofdstuk 4 gaat nader in op de kinderen die gediagnosticeerd zijn met mentale retardatie of taalstoornis. De relatie tussen de hersenvolumes en intellectueel functioneren is hier beschreven. Eerdere studies die (pre)adolescente kinderen met een normale ontwikkeling bestudeerden, lieten zien dat er een correlatie is tussen hersenvolume en intellectueel functioneren (tussen 0.20 en 0.28) (Wilke et al., 2003; Reiss et al., 1996). In onze groep van kinderen met mentale retardatie of taalstoornis vonden wij geen correlatie tussen intellectueel functioneren en intracranieel volume of totaal breinvolume. Wel vonden wij een correlatie van 0.74 tussen intellectueel functioneren en totaal breinvolume, na correctie voor intracranieel volume. Dit houdt in dat hersenvolume in relatie tot intracranieel volume 56% van de variantie in intellectueel functioneren verklaart in jonge kinderen met een ontwikkelingsstoornis. Een correlatie tussen intellectueel functioneren en hersenvolume, alleen na correctie voor intracranieel volume, suggereert dat er een effect van laag intellectueel functioneren op hersenvolume is dat ergens gedurende de ontwikkeling optreedt. De vroege ontwikkeling is waarschijnlijk normaal (gezien een normaal intracranieel volume), terwijl latere groei gehinderd is of later volumeverlies tijdens de ontwikkeling versneld of versterkt is.

De relatie tussen hersenvolume en intellectueel functioneren keert terug in hoofdstuk 5, waar ook de kinderen met autisme en PDD-NOS worden geïncludeerd. In dit hoofdstuk worden de hersenvolumes van de kinderen met ASD vergeleken met de hersenvolumes van de kinderen met een ontwikkelingsstoornis zonder ASD. Drie eerder gepubliceerde studies met jonge kinderen rapporteerden 5-10% grotere hersenvolumes in kinderen met ASD wanneer vergeleken wordt met kinderen met een ontwikkelingsachterstand of met normaalontwikkelende kinderen (Hazlett et

al., 2005; Sparks et al., 2002; Courchesne et al., 2001). Wij vonden geen verschillen in de gemeten breinvolumes (intracranieel volume, totaal breinvolume, ventrikelvolume, cerebellair volume, volumes van grijze en witte stof, hippocampaal volume en amygdala volume) tussen de groep kinderen met ASD en de controle groep van kinderen met een ontwikkelingsachterstand zonder ASD. Dat onze resultaten anders zijn dan eerder gepubliceerde resultaten, kan veroorzaakt worden door verschillen tussen de populaties die in de verschillende studies geïncludeerd zijn. Zo bleek dat de kinderen in onze studie veel radiologische abnormaliteiten lieten zien op de MR beelden (zie hiervoor ook hoofdstuk 2). Dit kan wijzen op een mogelijk andere etiologie met een zwaarder aangedaan brein. Ten slotte geldt dat, evenals in hoofdstuk 3, een controlegroep van normaal ontwikkelende kinderen niet aanwezig was, in tegenstelling tot in eerdere studies.
In vervolg op hoofdstuk 4, is de relatie tussen intellectueel functioneren en breinvolume bestudeerd bij de kinderen met ASD. De positieve correlatie tussen intellectueel functioneren en totaal breinvolume na correctie voor intracranieel volume eerder gerapporteerd voor de groep kinderen met een ontwikkelingsstoornis zonder ASD, kon niet gerepliceerd worden bij de kinderen met ASD. De afwezigheid van een significante correlatie tussen intellectueel functioneren en relatief breinvolume suggereert dat bij kinderen met ASD extra hersenvolume niet functioneel wordt geïntegreerd, wat wel het geval is bij kinderen met een ontwikkelingsachterstand. Men zou kunnen concluderen dat een relatieve vergroting van het brein niet leidt tot verbeterd functioneren voor patiënten met autisme.

In hoofdstuk 6 wordt een kleine samenvatting gegeven van de verschillende hoofdstukken en worden de resultaten met meer diepte besproken. In het algemeen kunnen we stellen dat we de resultaten van eerder gepubliceerde studies niet hebben kunnen repliceren. Bij het gebruik van spectroscopie vonden we geen verschillen tussen de groep met ASD-patiënten en de groepen kinderen met mentale retardatie en taalstoornissen. Wij menen dat dit veroorzaakt zou kunnen zijn door de kleine leeftijdsrange van onze patiënten en onze stringente correctie voor leeftijd en niveau van functioneren. Belangrijk is ook dat vooralsnog informatie ontbreekt over de biologische spreiding van metabolietenconcentraties in gezonde kinderen ongeacht welke leeftijd, laat staan in kinderen met een ontwikkelingsafwijking.

Met behulp van structurele MRI kwamen er geen verschillen naar boven in de hersenvolumes tussen de groep van kinderen met ASD en de groep kinderen met een andere ontwikkelingsstoornis. Dit zou veroorzaakt kunnen worden door verschillen tussen onze patiënten- en controlegroep en de groepen uit andere studies. Het merendeel van eerder gepubliceerde studies schenkt weinig aandacht aan mogelijke afwijkingen in de hersenen en zou daarmee subtiele radiologische afwijkingen over het hoofd kunnen zien. In hoofdstuk 2 hebben wij specifiek aandacht besteedt aan deze afwijkingen. Dit is de eerste studie sinds 1990 die hier aandacht aan besteedt (Piven et al., 1990). De relatie tussen intellectueel functioneren en hersenvolume was nog niet beschreven bij jonge kinderen met een ontwikkelingsstoornis. Wij vonden een positieve relatie tussen intellectueel functioneren en totaal breinvolume na correctie voor intracranieel volume, wijzend op een mogelijk afwijkende groei van het brein ten opzichte van het cranium in kinderen met een ontwikkelingsachterstand zonder ASD. Het ontbreken van een relatie tussen intellectueel functioneren en breinvolume in patiënten met ASD is niet onverwacht. Het was echter nog niet bestudeerd in jonge kinderen bij wie zowel brein als cognitie een grote ontwikkeling doormaken.

Er zijn nog enkele methodologische zaken die aandacht behoeven. Omdat het spectrum van autistische stoornissen zo breed is, is het moeilijk om de inclusie van patiënten te standaardiseren over verschillende (internationale) studies. Dit geldt met name voor de inclusie van erg jonge patiënten, voor wie sommige DSM-IV criteria niet gelden (Volkmar et al., 2005) en voor wie niet alle elementen van de ADOS en ADI geschikt zijn (Cox et al., 1999; Stone et al., 1999; Lord, 1995). Het is hierdoor moeilijker om de resultaten van studies met jonge kinderen onderling te vergelijken. Bovendien worden in studies met oudere patiënten vaker hoogfunctionerenden geïncludeerd, terwijl de geïncludeerd jonge kinderen met ASD vaak laagfunctionerend zijn (hoogfunctionerende kinderen worden vaak pas op latere leeftijd gediagnosticeerd). Dit beperkt vergelijkingen tussen leeftijdsgroepen en de generaliseerbaarheid van de resultaten van studies met oudere kinderen naar jonge groepen en vice versa.
Een tweede aspect is het veelvuldig samengaan van ASD en mentale retardatie. Syndromen met mentale retardatie zijn vaak geassocieerd met kleine hoofd-

omtrekken en kleine hersenvolumes. Autisme, daarentegen, wordt geassocieerd met grote hoofdomtrekken en grote hersenvolumes. Dat betekent dat, wanneer de kinderen met ASD ook mentaal geretardeerd zijn, de keuze voor een controlegroep erg belangrijk is voor de interpretatie van de resultaten. In de studies die zijn beschreven in dit proefschrift werd gebruik gemaakt van een controlegroep van kinderen met mentale retardatie en taalstoornis. Idealiter zou ook een groep van normaal ontwikkelende kinderen geïncludeerd moeten worden, maar dit is, zoals als eerder gezegd, vooralsnog in Nederland niet mogelijk.
Ten derde, uiteindelijk werden voor deze onderzoeken 48 kinderen met autisme, PDD-NOS, mentale retardatie of taalstoornis gescand. Veel moeite is gedaan om met name extra controlesubjecten te includeren. Echter, moeilijkheden met identificatie en scannen van deze kinderen beperkten onze mogelijkheden voor een grotere controlegroep. Dit kan onze power hebben beïnvloed.
Een laatste methodologisch aspect is de meting zelf. Is het mogelijk om breinvolumes en metabolieten accuraat en consistent te meten? De methodologie van volumetrische MRI is vergelijkbaar tussen de verschillende onderzoeksgroepen. Hoewel scansequenties en post processing wel enigszins verschillen, worden de resultaten in het algemeen beschouwd als betrouwbaar en reproduceerbaar. Voor spectroscopie ligt de zaak echter gecompliceerder. Verschillende voxelmetingen kunnen niet gemakkelijk met elkaar vergeleken worden, want de localisatie verschilt tussen studies, en metabole concentraties verschillen met localisatie en leeftijd van de patiënt. Belangrijker nog is het feit dat de biologische variatie in de concentraties onbekend is in gezond ontwikkelende kinderen, laat staan in kinderen met ASD.

Uiteindelijk hebben 34 kinderen uit het SOSO-project deelgenomen aan de studies in dit proefschrift. Het bleek niet mogelijk te zijn om dit onderzoek te beperken tot kinderen die geïdentificeerd zijn vanuit het bevolkingsonderzoek. Dat deze 34 kinderen wel een groot deel van de totale onderzochte groep uitmaken, kan invloed hebben gehad op de resultaten. Immers, kinderen die via een bevolkingsonderzoek zijn gediagnosticeerd met ASD kunnen mogelijk in gedrag of ontwikkeling verschillen van kinderen die gediagnosticeerd worden, nadat de ouders om hulp en diagnose hebben gevraagd.

Aanbevelingen voor toekomstig onderzoek

Kan MRI en MRS onderzoek gebruikt worden om de hersenontwikkeling van jonge kinderen met een atypische ontwikkeling te bestuderen? Wij geloven dat het, ondanks de beperkingen van verschillende groepen, de keuzes voor controlegroepen, en de grote verschillen tussen leeftijden, waardevol kan zijn om de hersenontwikkeling van jonge kinderen met ASD met MRI en MRS in kaart te brengen. Door beeldvorming van de hersenen is het mogelijk om meer duidelijkheid te scheppen over het tijdstip van ontwikkelingsafwijkingen, de locatie ervan en de mogelijke neurobiologische processen die eraan ten grondslag liggen. Het is echter wel van het grootste belang om de groepen patiënten zo homogeen mogelijk te houden. Bij voorkeur zouden de geïncludeerde patiënten zoveel mogelijk dezelfde leeftijd moeten hebben, alsmede hetzelfde niveau van intellectueel functioneren. En zoals voor alle beeldvorming van ontwikkeling geldt, verdienen longitudinale studies de voorkeur boven cross-sectionele studies. Voor meer homogeniteit zouden de kinderen dan bij een tweede meting (en derde en verder) opnieuw gediagnosticeerd en getest moeten worden om rekening te kunnen houden met verdere ontwikkeling van symptomen en intellectueel functioneren. Een genensensitief design, zoals een high-risk studie van broertjes en zusjes van ASD patiënten, zou met name informatief kunnen zijn, omdat dit de mogelijkheid biedt om de heterogeniteit in het fenotype te verkleinen.

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Curriculum vitae, list of publications, oral presentations and abstracts

Curriculum vitae

Mijke Zeegers werd op 14 september 1976 geboren in Helmond. In 1994 behaalde zij haar VWO diploma aan de Stedelijke Scholengemeenschap Scheldemond in Vlissingen. Datzelfde jaar startte ze haar studie psychologie aan de Universiteit Utrecht. In 1999 rondde zij deze studie af met biopsychologie als specialisatie. Na haar afstuderen ging ze werken als onderzoeksassistent bij de Neuroimaging groep van de afdeling Volwassenpsychiatrie van het Universitair Medische Centrum Utrecht. In 2000 startte zij haar eigen imaging onderzoek bij Kinder- en Jeugdpsychiatrie als AIO van het Rudolf Magnus Instituut voor Neurowetenschappen. De resultaten van dat promotieonderzoek zijn beschreven in dit proefschrift.

List of publications

Zeegers M, van der Grond J, van Daalen E, Buitelaar JK, van Engeland H. Proton magnetic resonance spectroscopy in developmentally delayed young boys with or without autism. *Journal of Neural Transmission,* in press

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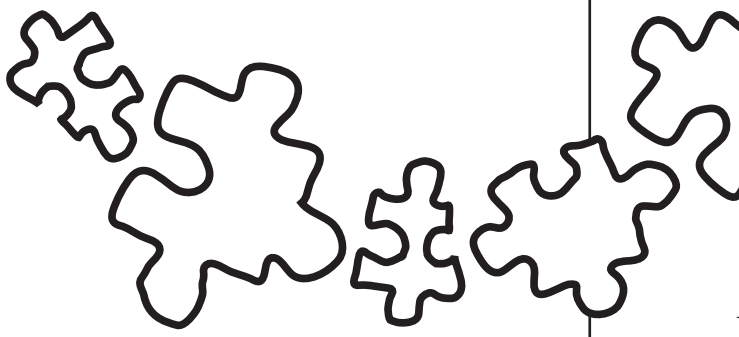
Zeegers M van der Grond J, van Daalen E, Buitelaar JK, van Engeland H. Proton spectroscopy in developmentally delayed young children with and without autism spectrum disorders. European Society for Child and Adolescent Psychiatry, October 2003, Paris, France

Dankwoord



I may not have gone where I intended to go,

but I think I have ended up where I needed to be.

Douglas Adams

Alle kinderen en hun ouders wil ik bedanken dat zij, met alle onzekerheid en onrust die zo'n uitgebreide medische check-up met zich mee brengt, toch wilden deelnemen aan mijn onderzoek. Zonder jullie zou dit proefschrift hier niet liggen.

Beste Herman, het was soms wat moeilijk om een half uurtje in je agenda te vinden, maar onze gesprekken maakten altijd veel helder en ze hielpen mij mijn prioriteiten weer netjes op een rijtje te zetten. Bedankt voor je nimmer aflatende interesse in mijn onderzoek.
Beste Jan, na een eerste periode waarin wij elkaar veel spraken, verminderde het contact toen je vertrok naar Nijmegen. Maar wanneer ik informatie nodig had of, in de laatste fase, commentaar op een stuk, dan reageerde je altijd snel en adequaat. Dank je wel voor je hulp bij het afronden van dit proefschrift.
Beste Jeroen, zonder jouw steun op kritieke momenten had ik nog veel langer doorgemodderd; bedankt voor het vlottrekken als ik weer vast zat. Ik heb veel van je geleerd; van scannen en artikelen schrijven tot je mening over hoe de wereld werkt. Bedankt voor je snelle en vaak ook laconieke reacties, voor je relativeringsvermogen en humor. Het was erg fijn om met je samen te werken.
Beste Hilleke, het gebeurde heel vaak dat ik je belde buiten werktijd of als jij net op het punt stond om weg te gaan. Toch kon je me dan uitgebreid te woord te staan. Je hebt van tevoren duidelijk gemaakt dat je weinig tijd had, maar als puntje bij paaltje kwam, was je er wel. Bedankt daarvoor.

Aan alle medewerkers van het AZU en WKZ die het mogelijk hebben gemaakt om mijn kinderen in te plannen, op te nemen en te onderzoeken: mijn grote dank. Met name wil ik noemen Gepke Visser (zelfs het invullen van formulieren maakte al dat ik het gevoel had dat er van alles kon gaan gebeuren), Regi, Gerdi, en de medewerkers van Dolfijn en het Sylvia Toth Centrum. Ook wil ik de anesthesisten en de laboranten van Radiologie bedanken. Mede door jullie was vrijdag-scandag altijd een feestdag.
Rutger Jan Nievelstein en Theo Witkamp wil ik bedanken voor hun bijdrage aan hoofdstuk 2. Na een pediatrisch neuroradiologie congres in Zwitserland was het mooi en leerzaam om de praktijk te zien.

Sarah, jeetje, waar te beginnen, meer dan wie ook heb jij heel veel rollen gehad in mijn promotie. Ik wil je bedanken voor de leuke tijd die we hadden als kamergenoten, voor je luisterend oor als ik mijn hart of verontwaardiging uit moest storten, voor je F1 binnen SPSS (samen bleken we er toch meer van te begrijpen), voor je hulp bij het schrijven (ik heb veel van je geleerd), voor het leiden van onze Niche en nu dan dat je mijn paranimf wilt zijn. Je bent voor mij de ultieme onderzoeker en mijn Niche-collega's boffen met zo'n geweldige co-promotor. Ik hoop dat we ook contact zullen houden nu ik uit Utrecht weg ben.
Lieve Esther, we zijn vriendinnen sinds je onder hypnose aria's zong en ik zou niet meer zonder je kunnen. We hebben de afgelopen jaren lief en leed gedeeld en je verfrissende blik op promotie en privé bleef me aan het denken houden. Of we nou met z'n tweeën, vieren of zevenen afspreken, het is altijd gezellig en altijd voel ik me daarna beter dan daarvoor. Ook al verhuizen we elke keer iets verder weg van elkaar, laten we elkaar blijven zien, bellen en mailen, want we zullen nooit uitgepraat raken.

Mijn mede-SOSOers, het was fijn om een deel van een groter project te vormen (alleen al om de frustraties over data en database te delen). Emma, bedankt dat je me vaak wou helpen om weer iets uit te zoeken over de patiënten. Samen queries maken bleek gewoon leuk en af en toe bijpraten was altijd leuk. Claudine, bedankt voor het geduldig beantwoorden van al mijn IQ-, PEP- en Mullenvragen. Zonder jouw hulp bij het gepluis in de database was in ieder geval hoofdstuk 4 er niet geweest. Fabiënne, je liet als eerste zien dat het mogelijk was om een SOSO promotie af te ronden. Dank je wel voor dat goede voorbeeld!

Lieve Manon, van thalamus en TRA (technology related anger) naar autisme, je bent een geweldige collega en vriendin. Het was een heerlijk idee dat ik niet de enige was die af en toe van stress liep te stuiteren. Laten we nu ook wat vaker afspreken voor leuke dingen, jouw bruiloft in Frankrijk was een heel goed begin!

En opeens was daar Niche, ons eigen kinderclubje. Lieve Hilde, Jiska, Marieke, Martijn, Nathalie, Sarah, Tim, Wouter en eventjes ook Dieter, bedankt voor jullie interesse in werk en ook privé. Het laatste jaar heb ik vrijwel alleen thuis gewerkt, maar de Niche-kamer was een warm bad om elke keer naar terug te keren. Wat kunnen collega's toch leuk zijn!
Alle morfo-ers van de afgelopen 7 jaar wil ik ook bedanken en dan speciaal natuurlijk Neeltje, voor alle structuur en stressbestendigheid, en Hugo, voor de essentiële bijdrage van het aanpassen van alle bestaande scripts aan mijn kleintjes. Je krijgt, geloof ik, nog een ijsje van me.
In de afgelopen jaren zijn er veel onderzoekers gekomen en gegaan, maar in het TAO heb ik ze allemaal leren kennen. Iedereen bedankt voor het aanhoren van alle problemen, voor het delen van jullie frustraties, voor de tips en trucs en vooral ook voor de gezelligheid. Mirjam, bijpraten lukte niet vaak, maar was wel heel fijn. Ik wil je heel veel succes wensen met de afronding van jouw promotie. Saskia wil ik met name nog even bedanken, want als kamergenoten hebben we natuurlijk veel gedeeld. Door jou en Sarah was onze kamer toch wel de gezelligste van de afdeling. Het was maar goed dat we er niet al te vaak tegelijkertijd waren.

In mijn leven buiten de promotie (al was ik er niet altijd van overtuigd dat het bestond) zijn er veel mensen geweest die heel belangrijk voor mij waren en zijn.
Lieve Sandra & Jeroen, met name in de eerste jaren van mijn promotie waren jullie er veel. Ik mis nog steeds het even binnen lopen voor een kop thee of tips voor het avondeten (chinees, friet?), en ik mis nog steeds dat we geen buren meer zijn. Zullen we gewoon weer vaker afspreken? Filmpje, chinees, friet?
Lieve Susanne, ergens afgelopen jaren hebben we weer contact opgenomen en al spreken we elkaar niet zo vaak als we zouden willen, je steun is heel belangrijk geweest. Meer dan wie ook weet jij hoe het afgelopen jaar was. Dank je wel dat je er was. Het is fijn om te weten dat iemand snapt hoe het voelt.

Corianne, het was voor de zwervers wel even wennen dat ik mailend op je stoepje zat. Dank je wel voor de laptop en internetverbinding. Je was een echte redder in nood. Nu ik klaar ben, is Rotterdam pas echt Blijdorp. Wie gaat er mee?
Beste Els, door alle bureaugebonden onderzoeksperikelen leek autisme vreemd genoeg soms heel ver weg. Bedankt voor je verhalen over Anna, ze maakten dat ik weer wist waar ik onderzoek naar deed.

LSZBZ (lieve schoonzussen, beste zwagers), bedankt voor de interesse die jullie vanaf het begin hebben getoond. Ik vond het ook heel leuk dat Pa altijd vroeg hoe het ging en naar welke congressen ik nu weer was geweest. Voor hem leek promoveren altijd zo vanzelfsprekend.
Lieve Femke, ik ben heel bij dat je mijn zusje bent. We zien elkaar eigenlijk veel te weinig. Nu dit af is, heb ik hopelijk meer tijd (en energie) om wat vaker bij jou en Martin langs te komen. Zullen we weer een keertje gaan stappen?
Lieve Klarien, lieve mam, ik geloof niet dat ik het ooit echt heb gezegd, maar jij bent in zoveel opzichten een rolmodel voor me. Ik heb bewondering voor je kracht en de manier waarop je altijd ook je eigen leven had naast je gezin. Dank je wel dat je me hebt geleerd dat ik de wereld aan kan en dat ik daarin mijn eigen weg kan vinden.
Lieve Ruud, lieve pap, ik mis je meer dan ik zeggen kan en meer dan ik had gedacht. Je hebt me zoveel mee gegeven en me zoveel geleerd. En net nu we op het punt stonden om alles te oogsten, ben je er niet meer. Dit boekje is voor jou, zoals beloofd die ochtend in de tuin. Ik had je hier zo graag op de eerste rij gehad. Ik wou zo graag dat je Cleo's opa langer had kunnen zijn. Ik weet dat je trots op me was. Ik ben ook zo trots op jou.

Liefste Cleo, als je aan het promoveren bent en veel thuis werkt, is het heel moeilijk om werk en privé gescheiden te houden. Maar toen jij er was, werd het heel makkelijk om mijn werkkamerdeur achter mij te sluiten. Alle problemen met analyseren en schrijven verdwenen als ik jou weer zag. Je bent altijd vrolijk en gezellig en ik ben heel gelukkig dat ik je mama ben.

Gabriël, mijn lief, ik had nooit geweten dat het leven zo mooi is, als ik jou niet had ontmoet. Elke dagdroom blijkt uit te komen en nog elke dag ben ik gelukkig dat jij van mij houdt en ik van jou. Je bent er altijd voor me en hoewel jij dat heel normaal vindt, ben ik er nog steeds heel blij mee. Dankjewel voor het goede leven (van pastis en Nazaré Pereira tot open haard en West Wing, het is altijd fijn), voor het meedenken, voor het coachen, voor alle verontwaardiging en voor je steun. Zonder jou was dit een ander boekje geweest en, belangrijker nog, was ik een ander mens geweest.

Life, just like I pictured it

Before you leave these portals
To meet less fortunate mortals,
There's just one final message
I would give to you
You all have learned reliance
On the sacred teachings of science,
So I hope, through life, you never will decline
In spite of philistine
Defiance
Do what all good scientists do

Experiment
Make it your motto day and night
Experiment
And it will lead you to the light
The apple on the top of the tree
Is never too high to achieve,
So take an example from Eve,
Experiment

Be curious,
Though interfering friends may frown
Get furious
At each attempt to hold you down
If this advice you always employ
The future can offer you infinite joy
And merriment,
Experiment
And you'll see

"Experiment" (uit "Nymph Errant") door Cole Porter