Development and Psychopathology, **14** (2002), 613–634
Copyright © 2002 Cambridge University Press
Printed in the United States of America
DOI: 10.1017.S0954579402003115

The neurobiological basis of autism from a developmental perspective

NATACHA AKSHOOMOFF, KAREN PIERCE, AND ERIC COURCHESNE
Children's Hospital Research Center and University of California

Abstract
Autism is a neurobiological disorder that is diagnosed through careful behavioral assessment in early childhood. In this paper, we review recent studies that have attempted to reveal the underlying causes of autism using a variety of techniques. Particular emphasis is placed on techniques that have been used by a number of different laboratories, including structural magnetic resonance imaging and postmortem studies of neuroanatomy. Neurobiological and neuropsychological data from individuals across a wide age range are examined from a neurodevelopmental perspective. We discuss how these recent advances have led us to develop a growth dysregulation hypothesis of autism. Finally, we discuss how this hypothesis may lead to new innovations in autism research.

The goal of research on the neurobiology of autism, particularly in our own laboratory, is to determine not only the underlying causes of the disorder but also its developmental trajectory. In autism, as in any neurodevelopmental disorder, this is by no means a straightforward problem. Autism is generally considered a polygenic or multifactorial disease; its genetic predisposition may be caused by the impact of many genes, each contributing a small effect on phenotype, or by the interplay of multiple environmental factors with multiple genes. Except for rare individual cases, which may or may not be similar in cause or syndrome to idiopathic autism, the causes leading to this disorder are, as yet, unknown.

Even as risk factors (genetic and/or teratogenic) for autism are identified, we may still lack an explanation of how they are responsible for specific neurodevelopmental abnormalities and subsequently how these abnormalities lead to the behavioral syndrome. To understand the neurobiological bases of autism, therefore, requires not only the discovery of "initial" triggering conditions (e.g., genetic mutations) but also the revelation of the multiple aberrant factors and processes that constitute the deviant epigenesis. To understand the causes of autism one must necessarily examine a variety of factors across multiple biological levels.

In this paper, we discuss the neurobiological bases of autism from a neurodevelopmental perspective. First, we briefly review neuroanatomical findings from older children, adolescents, and adults with autism. Second, we discuss recent magnetic resonance imaging (MRI) data from toddlers and preschoolers with autism. These data suggest that abnormal neural growth patterns early in development lead to an array of consequences in anatomical and functional organization. Third, we then discuss how this "growth dysregulation hypothesis" can be used to help interpret recent func-

The authors were supported by funds from the National Institute of Neurological Disorders and Stroke (R01 NS-19855), the National Institute of Mental Health (R01 MH-36840 and K01 MH01814), and Children's Hospital Research Center. The work in our laboratory is made possible by support from these funds, as well as the families who participate in our studies.

Address correspondence and reprint requests to: Dr. Natacha Akshoomoff, Laboratory for Research on the Neuroscience of Autism, Children's Hospital Research Center, 8110 La Jolla Shores Drive, La Jolla, CA 92037; E-mail: natacha@ucsd.edu.

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

tional MRI (fMRI) findings in high-functioning adults with autism. Fourth, we emphasize the importance of multiple levels of analysis interpreted within a developmental framework for understanding the pathobiology of autism.

Neuroanatomical Abnormalities

Among all the biological abnormalities identified thus far in autism, current evidence for neuroanatomical abnormality is the strongest. Postmortem and MRI studies have shown that most major brain structures are affected in autism, including the cerebellum, cerebral cortex, limbic system, corpus callosum, basal ganglia, and brain stem. Despite the growth of information pointing to multiple sites and types of neuroanatomical abnormality in autism, important and still controversial questions remain about most, though not all, findings. Moreover, as described later in this paper, little direct evidence exists regarding the brain in autism during early development, as postmortem evidence is obtained largely from adolescent and adult cases.

Recently, the brain weights of 21 postmortem cases of autism were compared to postmortem brain weights from more than 8,000 normal males and females (Courchesne, Müller, & Saitoh, 1999). When compared to the normal median weight, 17 of the 21 autism cases had normal brain weights, 1 case was microencephalic, and 3 autism cases were megaencephalic (2 was previously reported and 1 was a new case). Thus, brain weight is normal in most postmortem cases of autism. There are, however, rare cases of megaencephaly and possibly microencephaly.

Eight studies have reported either a slightly smaller brain volume in autism or no difference from normal average (Aylward, Minshew, Goldstein, Honeycutt, Augustine, Yates, Barta, & Pearlson, 1999; Creasey, Rumsey, Schwartz, Duara, Rapoport, & Rapoport, 1986; Gaffney, Kuperman, Tsai, & Minchin, 1989; Haznedar, Buchsbaum, Wei, Hof, Cartwright, Bienstock, & Hollander, 2000; Howard, 2000; Jacobson, Le Couteur, Howlin, & Rutter, 1988; Kates, Mostofsky, Zimmerman, Mazzocco, Landa, Warsofsky, Kaufmann, & Reiss, 1998; Rosenbloom, Campbell, George, Kricheff, Taleporos, Anderson, Reuben, & Korein, 1984). One MRI study reported increased whole brain volume (6% on average) in a group of 26 males (12–29 years of age) with autism (Piven, Arndt, Bailey, & Andreasen, 1996). Although this study showed significant enlargement in the temporal, parietal, and occipital lobes, another study reported that 43% of a sample of individuals with autism (6–32 years of age) had parietal lobe volume *loss* (Courchesne, Press, & Yeung–Courchesne, 1993).

We used MRI to report reduced cross-sectional size of the posterior corpus callosum in a large sample of patients with autism compared to a large sample of normally developing healthy control ("typical") subjects (Egaas, Courchesne, & Saitoh, 1995). This abnormality was replicated twice by Piven and colleagues (Manes, Piven, Vrancic, Nanclares, Plebst, & Starkstein, 1999; Piven, Bailey, Ranson, & Arndt, 1997a). No size reduction was found in the anterior corpus callosum.

In addition to the widespread cortical abnormalities noted in autism, several laboratories also reported abnormalities in subcortical structures, including the limbic system. A recent report of decreased amygdala volumes in autism (Pierce, Müller, Ambrose, Allen, & Courchesne, 2001) is consistent with Aylward et al. (1999) but inconsistent with Haznedar et al. (2000) and Howard (2000), who found either no difference or an increase in amygdala volume between autism and control subjects, respectively. This discrepancy across studies is likely attributable to differences in the constituency of the subject population: the Aylward et al. (1999) and Pierce et al. (2001) studies both used a sample of definite autism cases as diagnosed by multiple diagnostic instruments, whereas Haznedar et al. (2000) and Howard (2000) both utilized a mixed group of subjects with autism and subjects with Asperger disorder. It is important that, when data were distinguished by subject population, Haznedar et al. (2000) reported that left amygdalar volumes were *larger* in the subjects with Asperger disorder than in those with autism. Such a report not only implies that the neurobiology is different between groups but also suggests that mixing such

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

subjects during analyses may make finding true neurobiological differences difficult.

Piven and colleagues (Piven, Bailey, Ranson, & Arndt, 1998) did not find MRI evidence of hippocampal size abnormality in autistic adults. Despite this negative finding, there is postmortem evidence of abnormalities in the hippocampus and amygdala (Bailey, Luthert, Dean, Harding, Janota, Montgomery, Rutter, & Lantos, 1998; Bauman & Kemper, 1994; Kemper & Bauman, 1998; Raymond, Bauman, & Kemper, 1996). In a recent MRI study, Saitoh, Karns, and Courchesne (2001) found reduced size of the cross-sectional area of the dentate gyrus (the area dentata) in 59 subjects with autism (29 months to 42 years of age).

In contrast to the seemingly contradictory neuroanatomical findings in autism as described above, cerebellar pathology was found in 21 of 22 postmortem cases reported by five different research groups (Bailey et al., 1998; Bauman & Kemper, 1994; Fehlow, Bernstein, Tennstedt, & Walther, 1993; Ritvo, Freeman, Scheibel, Duong, Robinson, Guthrie, & Ritvo, 1986; Williams, Hauser, Purpura, DeLong, & Swisher, 1980). The first quantitative MRI studies identified abnormally reduced size of the cerebellar hemispheres (Gaffney, Tsai, Kuperman, & Minchin, 1987; Murakami, Courchesne, Press, Yeung–Courchesne, & Hesselink, 1989) and subregions within the cerebellar vermis in autistic children, adolescents, and adults (Courchesne, Hesselink, Jernigan, & Yeung–Courchesne, 1987; Courchesne, Yeung–Courchesne, Press, Hesselink, & Jernigan, 1988). Since then, there have been a number of additional studies conducted by different laboratories that reported significantly reduced size in one or another subregion of the cerebellar vermis (Carper & Courchesne, 2000; Ciesielski, Harris, Hart, & Pabst, 1997; Courchesne, Karns, Davis, Ziccardi, Carper, Tigue, Chisum, Moses, Pierce, Lord, Lincoln, Pizzo, Schreibman, Haas, Akshoomoff, & Courchesne, 2001; Courchesne, Saitoh, Yeung–Courchesne, Press, Lincoln, Haas, & Schreibman, 1994; Kates et al., 1998; Levitt, Blanton, Capetillo–Cunliffe, Guthrie, Toga, & McCracken, 1999). In the largest MRI study of autism ever conducted (more than 100 patients with autism and 100 control subjects), a reduction in the size of all vermis regions was reported for autistic infants, children, and adolescents (Hashimoto, Tayama, Murakawa, Yoshimoto, Miyazaki, Harada, & Kuroda, 1995). The greatest size reductions were seen in the youngest patients with autism. A recent radiological report shows that in some patients with autism, the cerebellar size reduction is so substantial that it is detectable by visual inspection alone (Miles & Hillman, 2000).

Despite the consistencies across these studies and the fact that the majority of neuroanatomical studies in autism focused on the cerebellum, some autism researchers reported that these results are "controversial" because other studies reported no significant group differences in vermis size. We discussed the possible explanations for these discrepancies in several articles (e.g., Akshoomoff, 2000; Courchesne, 1999; Courchesne, Townsend, & Saitoh, 1994); they include errors associated with measurement techniques (Garber & Ritvo, 1992; Garber, Ritvo, Chiu, Griswold, Kashanian, Freeman, & Oldendorf, 1989; Holttum, Minshew, Sanders, & Phillips, 1992), inadequate control groups (Holttum et al., 1992; Manes et al., 1999), and miscalculated cerebellar measures (Filipek, 1995; Piven, Saliba, Bailey, & Arndt, 1997). It is also important to note that the effect size is relatively small, and therefore the use of small sample sizes increases the likelihood of a Type II error. Data from two studies that reported no significant group differences in cerebellar vermis size (Kleiman, Neff, & Rosman, 1992; Piven, Nehme, Simon, Barta, Pearlson, & Folstein, 1992) were made available to our laboratory. Once outlier values from these small samples were taken into consideration and the data from the patients with autism were compared to age- and sex-matched, typically developing (normal) subjects, lobules VI and VII of the cerebellar vermis were found to be significantly smaller in the autism group (Courchesne, Townsend, & Saitoh, 1994). An examination of this data led to the discovery that some patients with autism, regardless of age, have an excessively large cerebellar vermis compared to age-matched typical subjects ("hyperplasia"). This was found to be the case in 2 of 15 autistic adults in the Piven et al.

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

(1992) study, 1 of 13 autistic children in the Kleiman et al. (1992) study, and 6 of 50 patients with autism between the ages of 2 and 40 years of age in data from our laboratory (Courchesne, Saitoh, et al., 1994). A discussion of these issues was also addressed by Piven and colleagues (Piven & Arndt, 1995; Piven, Saliba, Bailey, & Arndt, 1999).

The issue of a properly matched control group is obviously critical in studies of patient populations. A number of studies reported that there are regional volumetric changes with age and sexual dimorphism of brain structures in typically developing individuals (for a review, see Durston, Hulshoff, Casey, Giedd, Buitelaar, & van Engeland, 2001) and the size of some cerebral and cerebellar structures appears to be correlated with level of intelligence (Andreasen, Flaum, Swayze, O'Leary, Alliger, Cohen, Ehrhardt, & Yuh, 1993). Because approximately 75% of individuals with autism have an IQ below 70, several investigators argued that it is necessary to match subjects in the control group to the patients with autism for IQ in addition to age and sex (Filipek, 1995; Holttum et al., 1992; Kleiman et al., 1992; Piven, Saliba, et al., 1997). Therefore, some MRI studies included individuals with borderline IQs or a "developmental disorder" in their control group in order to match the autistic individuals on IQ. We believe that the key to understanding the neural basis of autism is to determine how the nervous system in individuals with autism differs from typically developing individuals (matched for age and sex). If one compares mentally retarded individuals with autism to individuals who are mentally retarded without autism, then one might learn which neuroanatomical abnormalities are specific to autism and which are more strongly linked to "mental retardation." However, this approach would require studying individuals with developmental disorders who do not have an identifiable cause for their mental retardation (e.g., Down syndrome) and would potentially be quite difficult, given the relatively limited number of ways in which the developing nervous system can be affected.

Other studies have only included individuals with autism who are not mentally retarded in order to compare their brain measurements to typically developing, IQ-matched control subjects. A recent study of 22 male individuals with autism (12–50 years of age) took the latter approach (Hardan, 2001). MRI data from these relatively high-functioning individuals (84–140 verbal IQ scores) were compared to typically developing males who were also recruited from the community. Area measurements of the anterior and posterior regions of the cerebellar vermis did not differ between the individuals with autism and the typically developing control subjects. However, the total volume of the cerebellum and the volume of the cerebellar hemispheres were significantly larger in the autism group. This result is interesting in light of our recent report of increased cerebellar white matter volume in preschoolers with autism and the potential link between neuroanatomical measures and autism severity (see the next section). Because this study included a relatively homogeneous group of autistic individuals, one must consider that the results may be limited to higher functioning children and adults with autism.

Some investigators argued that if a neural abnormality is more easily detected in mentally retarded individuals than those not mentally retarded, it is not an important feature in autism. Using this argument, and in light of the recent findings by Hardan and colleagues, one might conclude that decreased size of the posterior vermis is not a critical feature for understanding the neural basis of autism. We disagree. As we discuss below, we believe that by examining the relationship between these types of neuroanatomical variations and autism severity we can better understand brain–behavior relationships in autism.

Early Neural Growth Profiles in Autism

The majority of these MRI and postmortem studies have included adults with autism and have reported decreased size in a variety of structures. It is therefore unclear whether the reported neuroanatomical abnormalities are the developmental outcome of this lifelong disability or indicative of early pathology. MRI scanning in toddlers and preschoolers

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

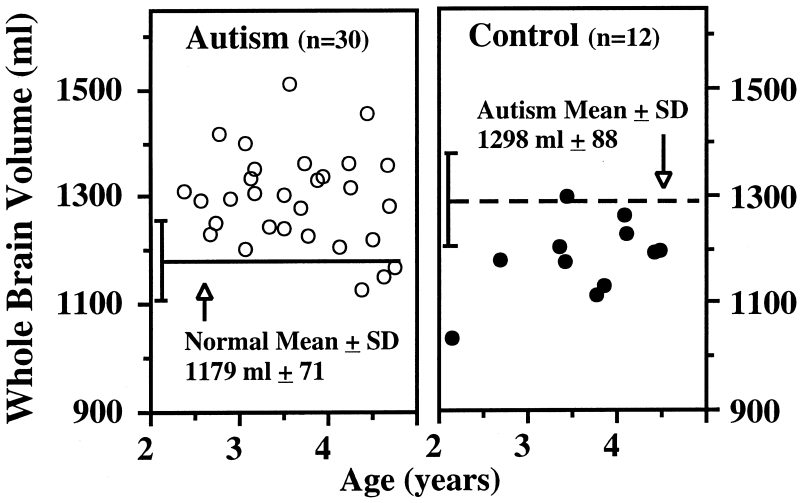


Figure 1. The whole brain volume in boys with autism and typically developing boys. Adapted from "Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study," by E. Courchesne et al., 2001, *Neurology, 57*, p. 249. Copyright 2001 by Lippincott, Williams & Wilkins. Adapted with permission.

with autism can provide data about the developmental time course and description for these abnormalities observed later in life.

In order to examine early brain development in autism, children need to be studied at as young an age as possible. In our recently completed study of 60 children with autism and 52 normal controls (ages 2–16 years), we found that brain volume appeared to be normal at birth (based on neonatal head circumference records) in children later diagnosed with autism (Courchesne, Bartholomeusz, & Karns, 2002; Courchesne et al., 2001). However, MRI data indicated that by 2–4 years of age, 90% of these autistic children had a brain volume larger than the average for the control group and 37% had brain volumes consistent with developmental macroencephaly (Figure 1). Cerebral white and gray matter were significantly larger than those found in the control group (normally developing, "typical" children), whereas older autistic children and adolescents did not have such enlarged gray and white matter volumes compared to the control group (Figure 2).

Cerebellar gray matter volume showed similar effects, but measurements from the youngest children with autism were more similar to age-matched control subjects whereas cerebellar gray matter volume was significantly smaller than controls in the older subjects. That is, because the cerebellum showed age-related increases in the control group but not in the autism group, the difference in cerebellar gray matter volumes between the autism and typical groups increased in magnitude with age. The cerebellar white matter volume showed an unexpected and striking abnormality. In the youngest subjects with autism, cerebellar white matter volumes were about 38% greater than in the control group. However, cerebellar white matter volumes increased little with age in autism, whereas in the typical group cerebellar white matter volume showed robust age-related increases, eventually reaching the volume seen in the youngest autistic children. The cerebellar vermis lobules VI and VII were reduced in size compared to the control group. In contrast to the control group, there were no age-related size increases in the vermis in the autism group.

The early cerebral volume enlargement noted in toddlers and preschoolers with autism appears to show an anterior to posterior gradient, which is largest at the frontal lobes and closer to typical in the occipital lobes (Carper, Moses, Tigue, & Courchesne, in press). The mean differences in frontal lobe volume com-

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

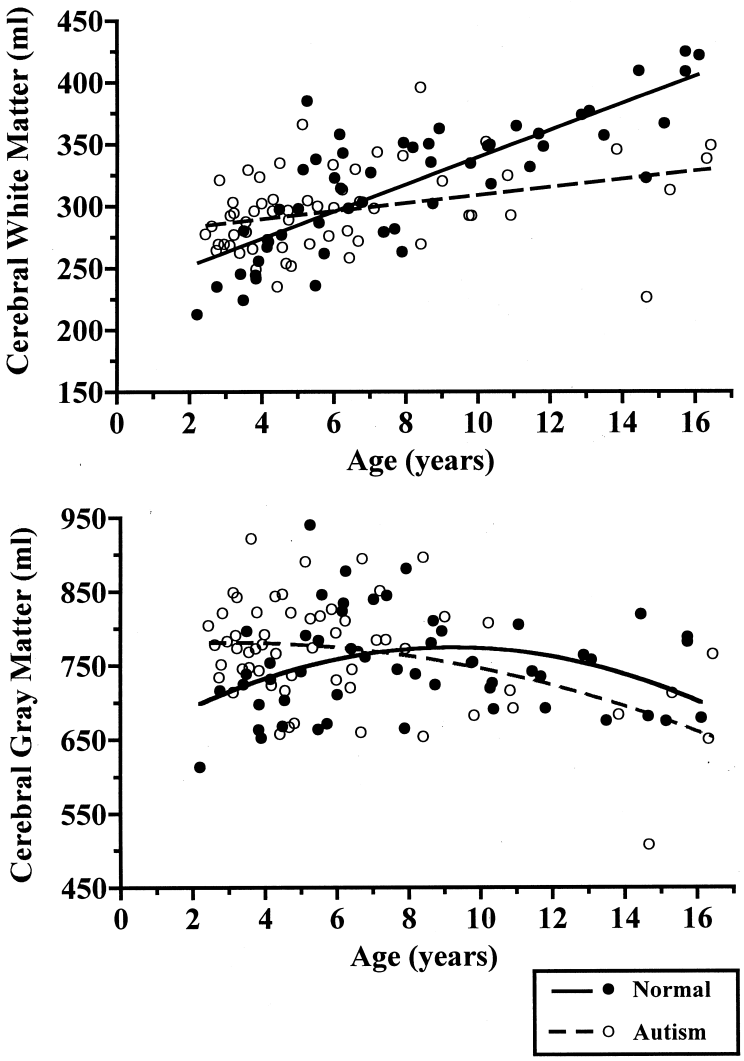


Figure 2. The cerebral gray and white matter volume by age. Adapted from "Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study," by E. Courchesne et al., 2001, *Neurology*, 57, p. 249. Copyright 2001 by Lippincott, Williams & Wilkins. Adapted with permission.

pared to the control subjects are illustrated in Figure 3. Interestingly, a statistically significant inverse correlation was found between the frontal lobe volume and the size of cerebellar vermis lobules VI and VII in children (ages 3–9) with autism (Carper & Courchesne, 2000). That is, the children with an abnormally large frontal lobe volume were those with an abnormally small cerebellar vermis area. Among same-age typical children, there was no significant relationship between frontal lobe volume and the area of cerebellar vermis lobules VI and VII. The correlation in autism suggests that there is a developmental link between these abnormalities.

The results from this MRI study are promising in terms of aiding our understanding of identifying early brain abnormalities in autism. This poses significant challenges because of the difficulty in accurately diagnosing autism in toddlers. That is, several studies demonstrated that standard diagnostic procedures were quite successful in identifying 2- and 3-year-olds who would continue to have

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

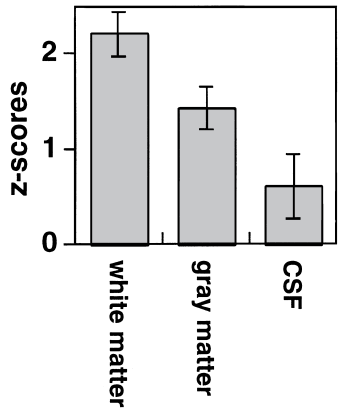


Figure 3. The frontal lobe volume enlargement in very young children with autism. Volumes were converted to *z* scores for each subject with autism (2.2–4.0 years of age) based on the means and standard deviations of the controls from the same age range. With this method, "0" on the *y* axis indicates the control mean, and "1" indicates 1 *SD* above the mean. The bar graph indicates the mean for the autism group; the error bars are 1 standard error of the mean. The frontal lobe white matter volume was significantly larger than normal and the frontal lobe gray matter showed an overall enlargement in autism. CSF, cerebrospinal fluid.

an autism spectrum diagnosis but there was less stability regarding the specific disorder on the spectrum, such as a firm diagnosis of autism versus Pervasive Developmental Disorder—Not Otherwise Specified (PDD-NOS; Cox, Klein, Charman, Baird, Baron–Cohen, Swettenham, Drew, & Wheelwright, 1999; Lord, 1995; Lord & Risi, 2000; Stone, Lee, Ashford, Brissie, Hepburn, Coonrod, & Weiss, 1999).

In our study, we used a multistep diagnostic procedure with children who initially received an MRI scan between 2 and 4 years of age. Children were recruited for the study if they had a clinical presentation that was consistent with PDD, including autism, as determined by at least two experienced diagnosticians and if they met criteria for autism on any one of the following: Autism Disorder Interview—Revised (ADI-R; Lord, Rutter, & Le Couteur, 1994), the Pre-Linguistic Autism Diagnostic Observation Scale (ADOS; DiLavore, Lord, & Rutter, 1995) or the ADOS—Generic (ADOS-G; Lord, Risi, Lambrecht, Cook, Levanthal, DiLavore, Pickles, & Rutter, 2000), and the Childhood Autism Rating Scale (CARS; Schopler, Reichler, DeVellis, & Daly, 1980). Use of the ADOS-G and CARS allowed inclusion of children who did not show significant evidence of restricted or repetitive behaviors due to their young age (Lord & Risi, 2000) but who did meet the social and communication criteria for autism. Children were reevaluated soon after age 5 by a team of psychologists, including Dr. Catherine Lord (a developer of the ADOS and the ADI-R), who derived a final best estimate diagnostic decision.

We recently compared MRI data from the 10 boys who had a final best estimate diagnosis of PDD-NOS from this study with 42 boys who were eventually diagnosed with autism (Akshoomoff, Lord, Davis, Ziccardi, Karns, Pizzo, Lincoln, Carper, Tigue, Courchesne, & Courchesne, 2001). The MRI data were obtained when the children were between 1.9 and 4.9 years of age and compared with typically developing boys of the same age. Of the children with a final diagnosis of autism, 12 had verbal and performance IQs of 70 or above ("higher functioning autism" or HFA group), and were thus matched to the children with PDD-NOS for verbal and performance IQ. We found that cerebellar white matter volume and anterior cerebellar vermis area was abnormally large in all three groups. Overall cerebellar volume was larger than normal in the HFA group. While the area of the posterior cerebellar vermis was smaller than normal in the lower functioning autism group, it was larger than normal in the HFA group. The results from this group of nonretarded preschoolers with autism is particularly interesting given the recent MRI study of the posterior fossa in high functioning 12- to 50-year-old males with autism (Hardan, Minshew, Harenski, & Keshavan, 2001).

Principles of Early Brain Development

The results from these studies provide evidence for the abnormal regulation of brain growth in autism. We hypothesize that this represents a pathological deviation of the mechanisms that normally regulate brain growth. Early brain development involves two major,

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

overlapping processes: progressive events and regressive events. The progressive events include neurogenesis, axon guidance, and formation of synapses and neurotransmitter receptors (Bayer, Altman, Russo, & Zhang, 1993; Huttenlocher & Dabholkar, 1997; Rakic, 1995). In fact, the vast majority of neurons are present by the seventh month of gestation (Rakic, 1995). In contrast, the growth of synapses, dendritic arbors, and fiber bundles largely takes place during early postnatal development. The elimination processes, or regressive events, include normal programmed cell death (apoptosis), axon pruning, and synapse elimination. Notably, there are significantly more neurons, axons, and synapses produced early in development than will be maintained in the later stages of brain development. Researchers have hypothesized that given the magnitude of these events, as well as individual differences in brain structure, a great proportion of these processes in the cerebral cortex must depend on experience (including the prenatal) rather than genetic prespecification (Killackey, 1990; O'Leary, 1992, 1989; Quartz & Sejnowski, 1998). That is, experience appears to have a major impact on progressive and regressive events. It is also important to note that some of these processes, such as synapse formation and elimination, continue throughout the life span as humans learn information, build new skills, and fail to rehearse certain behaviors.

The same overarching epigenetic principles that govern the normal process of neural self-construction and self-organization presumably also govern pathological development, in which aberrant factors and processes lead to misconstruction and misorganization. For example, when the *Otx-1* gene is eliminated in a "knock-out mouse" model, there is an abnormal retention of axonal exhuberance (Weimann, Zhang, Levin, Devine, Brûlet, & McConnell, 1999). An important principle to keep in mind is that at every time point, molecular, cellular, and functional conditions are the outcome of the conditions of the previous time point. Although it is convenient to think in terms of singular "causes" of an outcome condition (particularly a pathological outcome in neural organization), more often the outcome results from complex nonlinear interactions among multiple factors and processes (Cicchetti & Rogosch, 1996; Jessell & Sanes, 2000; Yeung–Courchesne & Courchesne, 1997). This makes it difficult to identify the cause or causes of a later abnormality in development and also helps to explain why individual cases can experience the same initial pathological events but have different functional outcomes.

Evidence of the Biological Time Course

Our MRI data provide evidence of brain growth abnormalities in toddlers and preschoolers with autism. Other pieces of biological evidence also provide information about the timing of pathology. These range from very early prenatal development (embryogenesis and the second trimester) to postnatal development, at least up through the preschool years.

A recent molecular assay finding provides data suggesting that abnormalities are present at the time of birth in autism. Nelson and colleagues (Nelson, Grether, Croen, Dambrosia, Dickens, Jeliffe, Hansen, & Phillips, 2001) reported abnormally elevated brain neurotrophins and neuropeptides (vasoactive intestinal polypeptide or VIP, brain-derived neurotrophic factor or BDNF, neurotrophin-4 or NT-4, and calcitonin gene-related protein or CGRP) in neonatal blood spots obtained retrospectively from neonates who later developed autism. This is the first potential neonatal marker of a risk for autism. However, it is not clear if these same substances are elevated during prenatal life and how long they continue to be elevated in postnatal life. It is also important to note that the samples were obtained from venous blood and therefore it is assumed, but not known, that these substances were also reduced in the brain. Further, elevations in VIP, BDNF, NT-4, and CGRP were also reported for the nonautistic mentally retarded group in that study (but not the patients with cerebral palsy). Although mental retardation is often associated with smaller than normal head size (e.g., microencephaly), head size was larger than normal in preschoolers with autism (Courchesne et al., 2001). However, VIP and

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

neurotrophins can influence the development of neurons and glia and the associated synapses in a number of ways, in terms of both production and elimination. It is therefore possible that although neonatal levels of these substances are elevated in both autism and mental retardation, this may lead to different consequences, depending on interactions with the genetic milieu for each child, as well as environmental differences.

At present, there are too few postmortem autism cases in the literature to provide definitive evidence of either the age at pathological onset or developmental trajectories for any specific brain structure. Only one postmortem case of autism under 6 years of age has been reported (Bailey et al., 1998). Nonetheless, neuropathological features evident in adult postmortem samples point to several different prenatal periods of onset. For example, agenesis of the superior olivary nuclei (Rodier, Ingram, Tisdale, Nelson, & Romano, 1996) is consistent with pathology that took place as early as the first trimester of prenatal development. Other features, such as cells in the inferior cerebellar peduncles that were arrested in migration (Bailey et al., 1998), suggest pathology during the prenatal period. Malformation of the inferior olivary nucleus (Bailey et al., 1998) and migration errors are clear signs of first trimester events, but these have only been seen in three autism postmortem cases. Other findings, such as irregular lamina disarray in small focal cerebral regions (Bailey et al., 1998) and reduced numbers of Purkinje neurons in the apparent absence of glial scarring (Bailey et al., 1998; Bauman & Kemper, 1994), may be indicative of later prenatal or early postnatal pathology.

Other reported pathological features, such as small cell size (Bauman & Kemper, 1994; Kemper & Bauman, 1998), increased cell packing density (Bauman & Kemper, 1994; Kemper & Bauman, 1998), and thickened cortices (Bailey et al., 1998), are not by themselves indicative of any particular age of onset. However, it is parsimonious to hypothesize that the onset of these types of abnormalities occurred during early neural development.

Cerebellar pathology is ubiquitous among published postmortem cases and across a large number of MRI studies of autism. Decreased Purkinje neuron numbers in postmortem cases is not accompanied by empty basket cells and the cerebellar folia do not show signs of atrophy (Bailey et al., 1998; Bauman & Kemper, 1994; Kemper & Bauman, 1998). These findings are indicative of earlier rather than later developmental age at the time of loss. In several autism cases, the inferior olivary nuclei (which are developmentally, structurally, and functionally intimately involved with cerebellar Purkinje neurons) were maldeveloped (Bailey et al., 1998). Neuronal migration errors also appeared in the inferior cerebellar peduncles (Bailey et al., 1998). In one case, Purkinje neurons were irregularly aligned (Bailey et al., 1998), which cannot be caused by some later postnatal event. Despite the reduced number of Purkinje neurons, the cerebellar cortex had clearly defined cortical lamina (granule, Purkinje, and molecular layers), even in the cases where there were large distances between single surviving Purkinje neurons (Bailey et al., 1998; Bauman & Kemper, 1994; Kemper & Bauman, 1998). Although Purkinje neuron loss or dysgenesis could occur prenatally along with olivary dysgenesis and the migration errors in the inferior cerebellar peduncle, observations of intact cerebellar lamina seem to argue against a massive dysgenesis or loss of Purkinje neurons before the final phases of granule cell proliferation and migration. Therefore, the possibility cannot yet be reasonably ruled out that Purkinje neuron loss could occur postnatally, perhaps in the first 1 to 2 years of life.

One recent postmortem study reported a reduction in antiapoptotic protein Bcl-2 in the cerebellum of five autism cases compared to controls (Fatemi, Halt, Stary, Realmuto, & Jalali–Mousavi, 2001). The authors hypothesized that dysregulation of this protein could be responsible for the observed tissue loss in the cerebellum in autism and reduced size of Purkinje neurons in autism (Fatemi, Halt, Earle, Kist, Realmuto, Thuras, & Merz, 2000). Bcl-2 is known to be differentially regulated during early gestation, perinatal development, and early adulthood. Although it is not known

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

if reduced levels of Bcl-2 in the adult brain are related to reduced levels in early development, this is certainly a possibility.

Growth Dysregulation Hypothesis

We believe that autism is a disorder of growth regulation. As described above, there is provocative evidence from postmortem and MRI studies supporting the idea of abnormal chronicity of neural growth in this disorder, including enlarged cerebral volume during the toddler years followed by widespread hypoplasia as individuals with autism reach adulthood.

There are many possible explanations for the increased cerebral gray and white matter volume and cerebellar white matter volume observed in early development in autism. One possibility is an increased number of neurons due to excessive sparing (i.e., decreased apoptosis) early in postnatal development or a delay in the timing of apoptosis. This may not necessarily be observed as increased brain volume or head circumference at birth. The time course of this effect could also differ across brain regions, just as the timing of normal apoptosis and synapse elimination differs across regions (Huttenlocher & Dabholkar, 1997). This could help to explain the abnormal enlargement of the frontal lobes in toddlers and preschoolers with autism but the closer to normal volume in other cerebral regions (Carper et al., in press). Another possibility is an abnormal retention of an excessive number of axonal connections. Again, this may be due to an excessive sparing of axons that are normally eliminated in early development or a delay in the timing of normal axonal elimination.

Neonatal elevations in VIP, BDNF, NT-4, and CGRP (Nelson et al., 2001) may contribute to abnormal neural growth, such as we observed in our MRI study of toddlers and preschoolers later confirmed to have a diagnosis of autism (Courchesne et al., 2001). These neurotrophic factors are known to increase cell survival, as well as increase the size and density of dendritic arbors (Lewin & Barder, 1996). While this process could thus lead to increased growth of neural elements in the cerebrum, paradoxically it could also result in a *loss* of Purkinje cells in the cerebellum. For example, when Morrison and Mason (1998) examined the role of BDNF and NT-4 on Purkinje cell development, they found that increased levels of BDNF and NT-4 resulted in a *decreased* overall number of Purkinje cells in the presence of granule cells (Morrison & Mason, 1998). This is also consistent with the results of our MRI study of toddlers and preschoolers with autism.

The impact of elevated brain neurotrophins and neuropeptides, however, could differ across children as a function of genetic background, timing, and experience. These differences may help to account for differences in the magnitude of neuropathology and functional outcome in children with pervasive developmental disorders. The greatest immediate importance of these brain growth findings, however, may be that abnormal levels of specific brain growth factors are clues to unraveling the biological mechanisms leading to brain maldevelopment in autism. As discussed earlier, growth rates appear slower throughout a variety of brain regions in children with autism after age 4, compared to typical children, who continue to show positive growth. Other brain regions are *reduced* in size at all ages examined. The identification of specific growth factor abnormalities may lead to a better understanding of which neurodevelopmental signaling pathways are abnormal in autism, and this in turn may point to candidate genes that could be ultimately responsible for the disorder.

If any or all of these pathological processes occur, then the postnatal construction of many areas of the brain may be occurring in a "willy nilly" fashion. That is, a number of neural circuits may be established in the first year or two based on abnormal preservation rather than experience-based competition. Connections that are not normally observed would therefore be maintained. This may lead to unusual patterns of behavior and the poor development of normal behaviors (such as language). As time goes on, some of these unusual connections, as well as some connections that would normally be strengthened through the process of normal behavioral development, would be eliminated. Thus, ironi-

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

cally, the "overgrowth" observed in early childhood might be a precipitating condition that ultimately leads to fewer than normal connections and decreased structure size.

There is a need for more neuropathological data to help explain these possibilities. For example, there is no evidence for an increased number of neurons in the adult autistic brain, and this seems unlikely, given the volumetric MRI data from older children and adults. There is also an absence of information about dendritic characteristics (i.e., arbor size and shape), numbers of synapses, axonal connections, and axonal arbors, and there have been no reports that include quantification of cell bodies in the cerebral cortex. In fact, postmortem information about the cerebral cortex in autism is currently limited to general findings (e.g., overall volume). Although this information would not give direct information about the timing of any observed abnormalities, it would help to establish how the nervous system is more directly affected.

Bringing Together Multiple Levels of Analysis

Data from the molecular, cellular, and macroscopic MRI levels all point to the same conclusion: there are a number of structures affected in autism that are associated with aberrant function. The pattern of behavioral abnormalities that define autism spectrum disorders is often associated with heterogeneity in symptom type, severity, and outcome. Recent studies, however, have begun to elucidate the pattern of brain–behavior relationships in autism. We will review recent findings from neuropsychological, neurophysiological, and functional neuroimaging studies and discuss how the results from these studies can be incorporated into a growth dysregulation hypothesis.

Findings from neurobehavioral and event-related potential (ERP) studies

A key feature of autism is attentional deficits (Bryson, Landry, & Wainwright, 1997; Casey, Gordon, Mannheim, & Rumsey, 1993; Ciesielski, Courchesne, & Elmasian, 1990; Courchesne, 1987; Courchesne, Akshoomoff, Townsend, & Saitoh, 1995; Courchesne, Kilman, Galambos, & Lincoln, 1984; Courchesne, Townsend, Akshoomoff, Saitoh, Yeung–Courchesne, Lincoln, James, Haas, Schreibman, & Lau, 1994; Dawson & Lewy, 1989; Pierce, Glad, & Schreibman, 1997; Schreibman & Lovaas, 1973; Waterhouse, Fein, & Modahl, 1996). Although deficits in attention are not unique to patients with autism and attentional deficits are not diagnostic of autism, it is hypothesized that deficits in attention may help to explain some of the social and cognitive deficits associated with the disorder.

Townsend and colleagues conducted several studies to investigate speed of attention orienting in autism and the brain systems that underlie these operations (Akshoomoff, Courchesne, & Townsend, 1997; Townsend & Courchesne, 1994; Townsend, Courchesne, & Egaas, 1996; Townsend, Courchesne, Singer–Harris, Covington, Westerfield, Lyden, Lowry, & Press, 1999; Townsend, Harris, & Courchesne, 1996). The performance of patients with autism was compared to that of patients with known brain damage in order to draw inferences about the neural systems affected in autism (Courchesne, Townsend, et al., 1994). Townsend's studies demonstrated that damage to the cerebellum leads to extremely slowed attention orienting. Patients with acquired cerebellar damage, as well as patients with autism (who have developmental abnormalities in the cerebellum), were significantly slower to detect a target presented shortly after an attentional cue than were control participants. However, both patient groups were as fast as control participants in detecting a target when they were given more time to orient their attention following a cue.

This neuropsychological approach was also used in a recent study of procedural learning in autism (Mostofsky, Goldberg, Landa, & Denckla, 2000). The experimental measures were ones that have been well studied in patients with specific types of brain damage. There are indications that separate operations in procedural learning are affected by damage to the frontal lobes, basal ganglia, and cerebellum. Functional brain imaging studies in normal subjects have also implicated a particular role

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

of the cerebellar–thalamic–frontal circuit in procedural learning. The results of the Mostofky et al. (2000) study indicated that deficits in procedural learning associated with autism are consistent with abnormalities in frontal–cerebellar circuitry.

Townsend, Harris, et al. (1996) also developed a task in which performance depends on speed of perceptual processing (target discrimination) rather than speed of a motor response (target detection). Just as in the 1994 study by Townsend and Courchesne, patients with autism were slow to orient attention to a cued location but significantly improved with a longer delay between cue and target. There was also a strong correlation ($r = .82$; $p < .01$) between the area of vermal lobules VI–VII divided by total brain volume (to control for overall brain size) and the degree of orienting deficit. Patients with acquired cerebellar damage showed similar deficits (Townsend et al., 1999).

The ERPs allow neural activity to be linked with behavioral performance and thus provide another avenue for investigating brain–behavior relationships in neurodevelopmental disorders. Stimulus overselectivity or overselective attention was reported for many patients with autism (Lovaas, Koegel, & Schreibman, 1979). Using electrophysiological and behavioral data, Townsend and colleagues found overselectivity in some adults with autism and broad, ungraded attention in others (Townsend & Courchesne, 1994; Townsend, Courchesne, et al., 1996; Townsend, Harris, et al., 1996). It appears that overselective attention is found primarily in individuals with autism who have parietal lobe volume loss (in addition to the cerebellar abnormalities previously described), whereas those with no parietal abnormality have abnormally broad, unfocused attentional responses. Only those with parietal cortex abnormalities had abnormally long reaction times to invalidly cued targets. In addition, reaction times were highly correlated with the degree of parietal lobe involvement.

Recent ERP evidence from our laboratory also suggests a disruption of spatial attentional networks in autism. The attention-related late positive ERP response (LPC) was investigated during visuospatial processing in a group of adults with autism (Townsend, Westerfield, Leaver, Makeig, Jung, Pierce, & Courchesne, 2001). The LPC is formed by the spatial and temporal overlap of multiple components. Three separate components were identified in this study, using Independent Components Analysis (Makeig, Westerfield, Jung, Covington, Townsend, Sejnowski, & Courchesne, 1999). The early frontal component (P3f), which may reflect attention orienting, was delayed or missing in adults with autism when attending to peripheral visual fields. The later parietal component (P3b), which may reflect context updating, was smaller in amplitude in adults with autism, regardless of attention location. It was hypothesized that damage to the cerebellum may have influenced both the frontal and parietal spatial attentional networks.

A recent study conducted in our laboratory included 17 children with a clinical presentation that was consistent with PDD who underwent MRI between 2.6 and 6.3 years of age (Harris, Courchesne, Townsend, Carper, & Lord, 1999). When the children were between 5 and 9 years of age ($M = 7.5$ years), they participated in an attention-orienting experiment similar to that used in a study of older autistic subjects (Townsend, Courchesne, et al., 1996). The degree of slowed orienting to visual cues was significantly correlated with the degree of cerebellar abnormality, but not with frontal lobe volume. Following the completion of this experiment, 11 of the 17 children were found to meet the diagnostic criteria for autism. The area of cerebellar vermal lobules VI–VII was significantly smaller in this subgroup, compared to the age-matched control group.

Attentional dysfunction in more naturalistic settings in young children with autism has also been linked to cerebellar abnormalities. In a recent study, Pierce and Courchesne (2001) found that when left alone to explore a room filled with boxes and hidden play items, children with autism explored minimal portions of their environment, in comparison to normal children. Further, autistic children with increased levels of cerebellar hypoplasia were more likely to fail to explore their environment than children with minimal or no cerebellar hypoplasia. No relationship between

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

these behavioral variables and other neuroanatomical measures, such as whole brain volume, was found.

These data demonstrate how one can test hypotheses about the relationship between specific cognitive operations and neurobiological data. These types of investigations breathe functional life into static data obtained from structural MRI and postmortem data and help us understand how variations in neuroanatomy correlate with variations in cognitive competence in a complex developmental disorder. One exciting feature of these studies is the possibility of identifying subgroups of patients on the basis of brain abnormalities (e.g., sulcal widening over the parietal cortex) and finding links with behavioral differences. The reverse has not yet been attempted in autism but this is also a possibility, using hypothesis-driven behavioral measures, appropriate neurobiological measures, well-defined patients, and adequate sample sizes.

Functional organization in autism

Although these types of brain–behavior studies in autism are beneficial in our understanding the impact of early neuroanatomical abnormalities in autism, such a correlational approach does not lend itself as readily to the investigation of the *systems* involved in complex behaviors. In combination with current knowledge on the neurobiology of autism that suggests that multiple structures and systems throughout the cerebrum and cerebellum are affected in autism, results from fMRI studies challenge the "one neural defect—one behavioral deficit" view of autism.

First, structures that are abnormal in autism, such as the frontal cortex and the cerebellum, normally participate in multiple neurobehavioral functions. For instance, fMRI and positron emission tomography studies of typical subjects show cerebellar activation during motor execution, sensory–motor learning, perceptual timing, attention, working memory, and language (Corbetta, 2000). Severe developmental defects in multifunctional structures such as the cerebellum or frontal cortex would thus be predicted to impair multiple functional domains, though not necessarily to equivalent degrees and in comparable ways in each domain or developmental period.

Second, many of the neurobehavioral functions that are abnormal in autism (e.g., social and language processing, executive functions, attention, motor execution, sensorimotor learning) activate multiple neural sites in typical individuals. For instance, imaging studies of typical subjects have shown frontocerebellar coactivation during semantic association (Petersen, Fox, Posner, Mintun, & Raichle, 1989) and working memory tasks (Casey, Cohen, O'Craven, Davidson, Irwin, Nelson, Noll, Hu, Lowe, Rosen, Truwitt, & Turski, 1998) and frontoparietal and cerebellar activation during visual spatial attention (Coull & Nobre, 1998) and problem solving tasks (Rao, Bobholz, Ham, et al., 1997).

Third, it is likely that neurodevelopmental defects in autism have an impact on the organization of neural networks. This was the conclusion of researchers who conducted longitudinal studies of children who suffered prenatal or perinatal unilateral brain damage (Bates, Thal, Trauner, Fenson, Aram, Eisele, & Nass, 1997; Elman, Bates, Johnson, Karmiloff–Smith, Parisi, & Plunkett, 1996; Stiles, 1998). That is, if the nervous system is affected before the functional pathways are fully formed, then the impact on behavior and function is different than if the damage occurs later in life. This may result in shrinkage, relocation, or fractionation of functional maps. It is also important to keep in mind that when infants are acquiring new skills, such as language, the systems involved in this process may be quite extensive compared to the systems that are ultimately primarily responsible for these skills later in life (Mills, Coffey–Corina, & Neville, 1994). However, it may be relatively easier to develop skills associated with a particular part of the brain following more restricted, early damage (e.g., a congenital unilateral stroke) than if the damage were of a different type (e.g., cell overgrowth or laminar disarray) or pervasive, such as in autism (Müller & Courchesne, 2000).

Fourth, autism appears to be a polygenic disorder (with the potential involvement of multiple genes), and each gene defect will likely have pleiotropic effects (Gelehrter &

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

Collins, 1990) on multiple brain structures and correspondingly multiple cognitive and sensorimotor domains.

Although there have been only a few published papers on fMRI in autism, several interesting trends have emerged. One of the most widely studied topics in autism relates to social processing. Two new fMRI studies (Pierce et al., 2001; Schultz, Gauthier, Klin, Fulbright, Anderson, Volkmar, Skudlarski, Lacadie, Cohen, & Gore, 2000) have shown that the robust phenomenon of fusiform gyrus activation in response to the presence of the human face is significantly diminished in autism. In combination with structural measures of regions hypothesized to be abnormal in autism (e.g., amygdala), we now have the ability to assay neuroanatomical structure, function, and behavior within the same individual. For example, results from the Pierce et al. study indicated that the neural sites that are active in typical subjects during face processing (fusiform gyrus, superior temporal sulcus, inferior occipital gyrus, and amygdala) were not the primary sites of activation in the participants with autism. Further, these functional deficits in autism were associated with a structural defect, namely, decreased amygdala volumes. Therefore, for the first time, direct evidence is available that suggests both structural and functional defects in systems involved in social processing in autism.

In addition to reports of decreased levels of activation in expected neural regions, fMRI has uncovered an interesting phenomenon in autism: increased activation in unexpected regions that appear to be unique for each individual with the disorder. For example, in the Pierce et al. study (2001), 100% of all of the typical subjects showed the strongest signal intensity change in response to pictures of faces in the posterior fusiform gyrus (i.e., the fusiform face area), whereas only one autistic subject exhibited a strong neural response in this region (Figure 4). Instead, the autistic participants in this study responded maximally to faces in various regions throughout the cerebrum (e.g., frontal lobe, occipital lobe) and cerebellum. As another example, Müller and colleagues (Müller, Pierce, Ambrose, Allen, & Courchesne, 2001) noted that during a simple finger movement task, individual functional maps across typical subjects showed consistent and robust activation in the contralateral primary sensorimotor cortex, with additional activation in the premotor and/or supplementary motor areas. In the autism group, however, the spatial consistency of such motor cortex activation was reduced, with several autistic subjects failing to show significant activation in the contralateral motor cortex at all (Figure 5). Further, such subjects showed significant activations in posterior neural regions not typically associated with this type of task, such as the superior parietal lobe and precuneus.

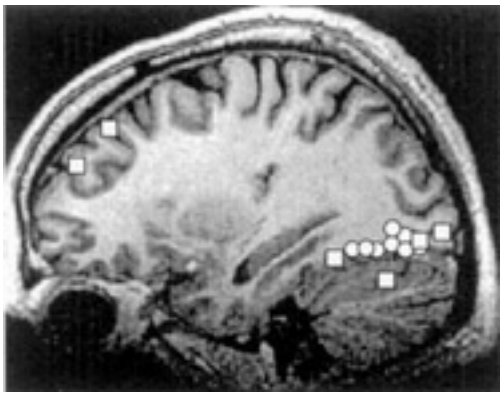


Figure 4. The regions of maximum signal intensity for six autistic and eight normal control subjects during a face perception task. Each symbol represents an activation "hot spot" for a single subject as overlaid on a sagittal image. For purposes of illustration, peak activations were collapsed from the left to right axis on the sagittal image. (□) Autism and (○) normal control. Note that the region with the highest signal intensity change between experimental and control conditions for (○) *all* normal control subjects is located within the fusiform gyrus in contrast to (□) hot spots across the cerebrum and cerebellum for autistic subjects. Adapted from "Face processing occurs outside the fusiform 'face area' in autism: Evidence from functional MRI," by K. Pierce, R.-A. Müller, J. Ambrose, G. Allen, & E. Courchesne, 2001, *Brain, 124,* p. 111. Copyright 2001 by Oxford University Press. Adapted with permission.

Relationship to a growth dysregulation hypothesis

Such a pattern of atypical functional maps observed during neuroimaging studies, including

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

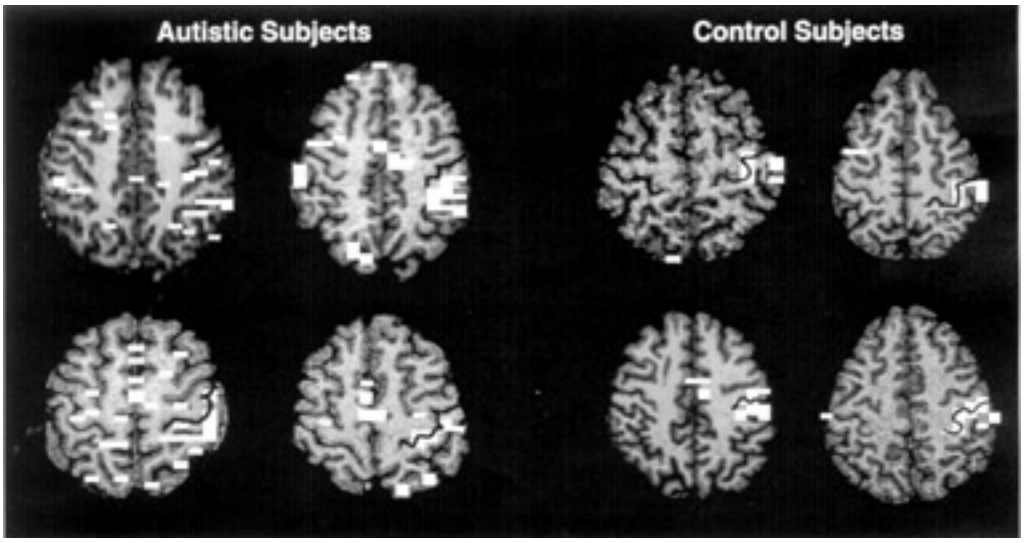


Figure 5. Axial slices showing activations ($p < .001$; uncorrected) for four autistic subjects (left) and four normal control subjects (right), overlaid in white onto individual structural magnetic resonance images in native space. The slices are selected to show the major activation cluster in the perirolandic cortex. The central sulcus (contralateral to the side of movement) is indicated by a black line. Each normal control subject showed the most robust activation cluster in the pre- and postcentral gyri along the central sulcus. Even though most autistic patients also showed such perirolandic activations, there were several types of atypical activation patterns during the motor task such as activation exclusively posterior to the central sulcus or overall scattered activation. Adapted from "Atypical patterns of cerebral motor activation in autism: A functional magnetic resonance study" by R.-A. Müller, K. Pierce, J. B. Ambrose, G. Allen, & E. Courchesne, 2001, *Biological Psychiatry, 49,* p. 670. Copyright 2001 by Elsevier Science Inc. Adapted with permission.

scattered and unique patterns of functional activity, is consistent with a growth dysregulation hypothesis in autism. Early widespread neuroanatomic defect, such as cerebral overgrowth in combination with the specific regions of hypoplasia (e.g., hippocampus and cerebellum) noted in the young autistic brain, would likely lead to abnormalities in the development of functional systems. Evidence from developmental neurobiology shows that when the developing brain sustains significant neural insult precipitated by environmental or genetic factors, the typical balance of competition that mediates functional organization may be altered (Kolb & Whishaw, 1989). This can result in atypical organization (including abnormally reduced, expanded, scattered, and lateralized representations) and atypical functional activity. The "plasticity" commonly associated with the developing brain may thus produce arrangements that successfully compensate for the structural defect, but it may also produce arrangements that do not. Perhaps this partially explains the different degrees of impairment among autistic individuals. If the developing autistic nervous system abides by well known principles of competition and neuroplasticity in the face of neural defect, then abnormal functional activity and organization would be present in the developed autistic brain. Moreover, because autism involves multiple types and sites of neural defect (e.g., a thickening of cerebral cortex in some postmortem cases; neuroanatomic pathology in the cerebellum in 19 of 20 postmortem cases), it is likely that abnormal functional activity and organization will affect different structures in different ways. Because many of the structures that are affected in autism mediate the development of more than one function and typical development entails competition between functions (or functional inputs), the imbalance created by various types and sites of neural defects in autism may dif-

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

ferentially affect the cerebral and cerebellar organization of different functional domains, with greater abnormalities for some functions than for others.

Although there are many potential applications of fMRI research both independently and in concert with research methods at other levels of analysis (e.g., structural MRI) there are several notes of caution.

First, many fMRI studies have utilized mixed subject groups that include both subjects with autism and subjects with Asperger disorder. It is currently unknown whether such groups represent etiologically distinct or similar disorders because of the paucity of neurobiological studies on Asperger disorder. One study suggests that autism and Asperger disorder may differ in some aspects of neuroanatomic abnormalities (Haznedar et al., 2000). Asperger disorder is categorized as a separate pervasive developmental disorder in the DSM-IV, and the main distinction from autism is the absence of qualitative impairments in communication and the absence of clinically significant delays in language, cognitive development, and adaptive behavior (other than social interaction). It is therefore imperative that individuals are carefully evaluated for early developmental history (e.g., using the ADI-R) in order to accurately distinguish between a diagnosis of Autistic disorder and Asperger disorder. The DSM-IV specifies that if a patient meets the criteria for another specific PDD (such as Autistic disorder), s/he cannot be diagnosed with Asperger disorder. Note that if an individual did not have clinically significant general delays in language development, s/he could still meet the criteria for autism. If these points are not taken into consideration, it is conceivable that high-functioning individuals who do meet the criteria for autism could be incorrectly diagnosed as Asperger disorder (Klin & Volkmar, 1997). This is a critical issue for fMRI researchers because typically only high-functioning individuals will be able to carry out the experimental demands required for fMRI experiments while also lying still for periods of time (particularly children). If patients are carefully evaluated and researchers decide to include patients with autism as well as patients with Asperger disorder, fMRI results from these patients should be analyzed separately and presented as such in order to increase our understanding of how these disorders may be similar and different.

Second, patterns of cerebral vasculature may be different in autism, and thus contribute to atypical patterns of functional activity. Perfusion studies have, in fact, noted hypometabolism in most cortical regions, including the frontal (Zilbovicius, Garreau, Samson, Remy, Barthelemy, Syrota, & Lelord, 1995) and temporal (Zilbovicius, Barthelemy, Belin, Remy, Poline, Mangin, Syrota, & Samson, 1998).

Third, the successful interpretation of functional neuroimaging results relies on its accurate integration with structural data. Currently, the majority of neuroimaging studies utilize a technique in which data are transformed into a particular set of stereotaxic coordinates (e.g., Talairach space). This approach assumes that the underlying neuroanatomy of constituents in a particular group is similar. As mentioned above, autism is a disorder with neuroanatomic abnormalities in diverse regions throughout the cerebrum and cerebellum.

Our Conclusions

Anatomical abnormalities in autism result from the developmental dysregulation of brain growth, the causes of which are genetic defects that produce detectable abnormalities in molecular brain growth factors.

According to this hypothesis of a progressive abnormality in brain growth in autism, there may be pathologic timing in the initiation of neuronal and glial growth as well as pathological timing in the cessation of growth. The result would be *premature overgrowth* in some structures (e.g., enlarged cerebral volumes, abnormal cerebral cortical thickening, excess numbers of neurons or glia) and *reduced growth or excessive cell loss* in other structures (e.g., reduced limbic and cerebellum volumes, too few cells generated, small cell size, reduced dendritic arborization, neuron loss in the limbic and cerebellar regions, cells arrested in migration). Other aspects of growth may look like overgrowth on MRI scans but have an alternative explanation. For example, a recent study of myelin in autism found that the biochemical composition of

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

myelin was altered and its compaction was delayed, which may account for increased white matter volume found via MRI (Koul, 2001). Koul hypothesized that the abnormal compaction of myelin in early development in autism may partially account for increased white matter volume on MRI scans (Courchesne et al., 2001).

Because normal growth is the result of a successful balance in the timing of gene-regulated induction and termination of growth signals (proliferation, migration, etc.), as well as the induction and termination of apoptosis, a failure of this balance would result in abnormal anatomy. The unusual pattern of hyperplasia in the frontal cerebral cortex and hypoplasia in the limbic structures and cerebellum found in autism suggest such failure.

A number of factors can tip this balance along the signaling pathways between the regulating genes and the growth outcome. For example, excessive or reduced levels of a required neuropeptide or neurotrophin for a specific growth step, erroneous timing or site of the appearance of a neurotrophin, and/or the failure of feedback signals to indicate its presence to trigger the next growth step can all create an imbalance. Neuropeptides and neurotrophins that are elevated in persons who later develop autism (Nelson et al., 2001) and a reduction of GABA-A receptors in the brain (Blatt, Fitzgerald, Guptill, Booker, Kemper, & Bauman, 2000) may be markers indicating such imbalance and, as such, could be used as signposts leading toward an identification of abnormal signaling pathways and affected growth-regulating genes.

We also hypothesize that the anatomic heterogeneity that has been consistently demonstrated in autism may reflect individual differences in levels of growth factors. These different levels may reflect either different forms of defect in a given key gene or the involvement of different combinations of genes within a signaling pathway in different autistic individuals. For example, Bailey et al. (1998) reported thickened cortex in some cases of autism and laminar disarray in the cortex in others; all cases had Purkinje cell loss. These different patterns of cortical growth and organization may be indicative of one process (or processes) in autism, and the Purkinje cell loss may be indicative of another more common process.

Implications and Future Directions

Future directions for MRI research

We believe that studying children with autism as early as possible will provide clues to the underlying neural abnormalities that are much more difficult to disentangle from experience in the older child or adult. Our recent MRI studies provide striking evidence in this regard. We see a potential *gradient* in growth pattern abnormalities from our MRI data of children who were scanned at as young as 30 months of age. At the earliest ages examined, there is an overgrowth of cerebral volume and gray matter as well as cerebral and cerebellar white matter, compared to normal. This difference diminishes with age but could obviously have lasting effects. In a subset of our subjects that have been examined to date, we have also found that excessive cerebral growth appears to be largest in the frontal lobes in the youngest children examined, with different patterns within frontal lobe regions (Carper & Courchesne, 2002; Carper et al., in press). It also appears that the posterior cerebellar vermis and the volume of cerebellar gray matter are typically smaller than in age-matched healthy control subjects at the earliest ages examined.

With these unusual growth patterns, it will be important to determine if there are behavioral distinctions related to these phenomena. That is, in what ways do children with a final outcome diagnosis of PDD-NOS differ from children with autism? Are there differences in the magnitude of early abnormalities between the children with autism who are higher functioning after age 5 compared with those who are lower functioning after age 5? We are currently examining these questions in our existing data set and in new studies in our laboratory. As described above, combining anatomical data with behavioral data helps to answer questions about brain–behavior relationships in autism. There is little data available in this regard for toddlers and preschoolers, and, as such, this population will be the focus of new studies in our laboratory.

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

Future directions for functional imaging research

At this relatively macroscopic level, multiple neuroimaging techniques, such as fMRI, MRI, diffusion tensor imaging (DTI), and perfusion imaging or arterial spin labeling, when utilized in *combination* with one another, will serve as a powerful analytic tool for the study of neural development in autism. For example, DTI makes use of the random motion of water molecules to create a map of fiber tracks in the living brain. The greatest benefit of DTI will be in detecting long-term changes in cerebral connections in autism across several ages during development. This will be invaluable for elucidating issues regarding the timing and progression of neural maldevelopment, as well as understanding more directly, how defects in one neural region may interact with, or contribute to, defects in other neural regions. This will be particularly important in combination with structural volumetrics, obtained with MRI when interpreting findings of corelationships between structures, such as the inverse correlation between frontal lobe and cerebellar vermal lobules VI–VII volumes noted by Carper and Courchesne (2000).

Concluding remarks

The future of autism research will undoubtedly continue and extend the use of multidisciplinary conceptual and analytical techniques. The development of animal models of autism, in particular, will be crucial for validating genetic or nongenetic explanations, as well as testing the direct impact of certain biological and behavioral treatments. Animal models of any disorder rely on the availability of brain–behavior linkage data to be modeled, and research that continues to elucidate our understanding of the neural substrates involved in the symptomatology of autism will be pivotal toward this aim. In fact, the advocated combined behavioral, neurofunctional, and neuroanatomical approach is consistent with the types of approaches currently taken in the study of animal mutants and knockouts, potentially facilitating the translation of information from these studies to future autism animal model studies.

We also anticipate that the combination of neuroimaging techniques, such as fMRI and ERPs, with cognitive and behavioral measures will help to sharpen diagnostic distinctions, not only between individuals with autism and typically developing individuals, but also between different subgroups within the disorder of autism (the "autism spectrum"). Along these lines, we recommend that researchers try to obtain multiple measures within the same groups of subjects in order to analyze heterogeneity, as well as similarities, and work toward maximal integration of information. Ultimately, we hope that the biological information obtained will influence our behavioral diagnoses and descriptions, and vice versa.

In the future we may be able to identify biological markers of autism, which, in turn, could be used to more accurately diagnose affected children at a very early age. The identification of biological markers will also help to reveal the underlying causes of this disorder, thereby also leading to more effective, "tailor-made" treatments, both behavioral and biological. A multidisciplinary approach is essential to achieving these goals. Recent exciting advances in the neurobiology of autism have involved these critical collaborations between behavioral and biological scientists. As we have discussed in this paper, it is only through bringing together information from multiple levels that we can hope to better understand as complicated a neurodevelopmental disorder as autism.

References

Akshoomoff, N. (2000). Neurological underpinnings of autism. In A. M. Wetherby & B. M. Prizant (Eds.), *Autism spectrum disorders: A transactional developmental perspective* (pp. 167–190). Baltimore, MD: Paul H. Brookes Publishing.

Akshoomoff, N. A., Courchesne, E., & Townsend, J. (1997). Attention coordination and anticipatory control. *International Review of Neurobiology, 41*.

Akshoomoff, N., Lord, C., Davis, H. R., Ziccardi, R., Karns, C., Pizzo, S., Lincoln, A. J., Carper, R. A., Tigue, Z. D., Courchesne, R. Y., & Courchesne, E. (2001). *MRI results from preschoolers with pervasive*

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

developmental disorders. Paper presented at the International Meeting for Autism Research, San Diego, CA.

Andreasen, N. C., Flaum, M., Swayze, V., O'Leary, D. S., Alliger, R., Cohen, G., Ehrhardt, J., & Yuh, W. T. (1993). Intelligence and brain structure in normal individuals. *American Journal of Psychiatry, 150,* 130–134.

Aylward, E., Minshew, N., Goldstein, G., Honeycutt, N., Augustine, A., Yates, K., Barta, P., & Pearlson, G. (1999). MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology, 53,* 2145–2150.

Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M., Rutter, M., & Lantos, P. (1998). A clinicopathological study of autism. *Brain, 121,* 889–905.

Bates, E., Thal, D., Trauner, D., Fenson, J., Aram, D., Eisele, J., & Nass, R. (1997). From first words to grammar in children with focal brain injury. *Developmental Neuropsychology, 13,* 275–343.

Bauman, M. L., & Kemper, T. L. (1994). Neuroanatomic observations of the brain in autism. In M. L. Bauman & T. L. Kemper (Eds.), *The neurobiology of autism* (pp. 119–145). Baltimore, MD: Johns Hopkins University Press.

Bayer, S., Altman, J., Russo, R., & Zhang, X. (1993). Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology, 14,* 83–144.

Blatt, G., Fitzgerald, C., Guptill, J., Booker, A., Kemper, T., & Bauman, M. (2000). *Reduced GABAA receptors in the hippocampal formation of individuals with autism* [Abstract]. Paper presented at the Society of Neuroscience (http://sfn.ScholarOne.com/itin).

Bryson, S. E., Landry, R., & Wainwright, J. A. (1997). A componential view of executive dysfunction in autism. In J. A. B. J. T. Enns (Ed.), *Attention, development, and psychopathology* (pp. 232–259). New York: Guilford Press.

Carper, R. A., & Courchesne, E. (2000). Inverse correlation between frontal lobe and cerebellum sizes in children with autism. *Brain, 123,* 836–844.

Carper, R. A., & Courchesne, E. (2002). *Localized enlargement of the frontal lobe and correlations with cerebellar hypoplasia in early autism*. Unpublished manuscript.

Carper, R. A., Moses, P., Tigue, Z. D., & Courchesne, E. (in press). Cerebral lobes in autism: Early hyperplasia and abnormal age effects. *Neuroimage.*

Casey, B. J., Cohen, J. D., O'Craven, K., Davidson, R. J., Irwin, W., Nelson, C. A., Noll, D. C., Hu, X., Lowe, M. J., Rosen, B. R., Truwitt, C. L., & Turski, P. A. (1998). Reproducibility of fMRI results across four institutions using a spatial working memory task. *Neuroimage, 8,* 249–261.

Casey, B. J., Gordon, C. T., Mannheim, G. B., & Rumsey, J. M. (1993). Dysfunctional attention in autistic savants. *Journal of Clinical and Experimental Neuropsychology, 15,* 933–946.

Cicchetti, D., & Rogosch, F. A. (1996). Equifinality and multifinality in developmental psychopathology. *Development and Psychopathology, 8,* 597–600.

Ciesielski, K., Harris, R., Hart, B., & Pabst, H. (1997). Cerebellar hypoplasia and frontal lobe cognitive deficits in disorders of early childhood. *Neuropsychologia, 35,* 643–655.

Ciesielski, K. T., Courchesne, E., & Elmasian, R. (1990). Effects of focused selective attention tasks on event-related potentials in autistic and normal individuals. *Electroencephalography and Clinical Neurophysiology, 75,* 207–220.

Corbetta, R. N. (2000). Imaging cognition II: An empirical review of 275 PET and fMRI studies. *Journal of Cognitive Neuroscience, 12,* 1–47.

Coull, J. T., & Nobre, A. C. (1998). Where and when to pay attention: The neural systems for directing attention to spatial locations and to time intervals as revealed by both PET and fMRI. *Journal of Neuroscience, 18,* 7426–7435.

Courchesne, E. (1987). A neurophysiological view of autism. In E. Schopler & G. B. Mesibov (Eds.), *Neurobiological issues in autism* (pp. 285–324). New York: Plenum Press.

Courchesne, E. (1999). An MRI study of autism: The cerebellum revisited. *Neurology, 52,* 1106–1107.

Courchesne, E., Akshoomoff, N. A., Townsend, J., & Saitoh, O. (1995). A model system for the study of attention and the cerebellum: Infantile autism. *Electroencephalography and Clinical Neurophysiology, Supplement, 44,* 315–325.

Courchesne, E., Bartholomeusz, H., & Karns, C. (2002). *MRI and clinical evidence of a postnatal overshoot in head and brain size in autism*. Manuscript submitted for publication.

Courchesne, E., Hesselink, J. R., Jernigan, T. L., & Yeung–Courchesne, R. (1987). Abnormal neuroanatomy in a nonretarded person with autism. Unusual findings with magnetic resonance imaging. *Archives of Neurology, 44,* 335–341.

Courchesne, E., Karns, C., Davis, H. R., Ziccardi, R., Carper, R., Tigue, Z., Chisum, H. J., Moses, P., Pierce, K., Lord, C., Lincoln, A. J., Pizzo, S., Schreibman, L., Haas, R. H., Akshoomoff, N., & Courchesne, R. Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology, 57,* 245–254.

Courchesne, E., Kilman, B. A., Galambos, R., & Lincoln, A. J. (1984). Autism: Processing of novel auditory information assessed by event-related brain potentials. *Electroencephalography and Clinical Neurophysiology, 59*(3).

Courchesne, E., Müller, R.-A., & Saitoh, O. (1999). Brain weight in autism: Normal in the majority of cases, megalencephalic in rare cases. *Neurology, 52,* 1057–1059.

Courchesne, E., Press, G. A., & Yeung–Courchesne, R. (1993). Parietal lobe abnormalities detected with MR in patients with infantile autism. *American Journal of Roentgenology, 160,* 387–393.

Courchesne, E., Saitoh, O., Yeung–Courchesne, R., Press, G. A., Lincoln, A. J., Haas, R. H., & Schreibman, L. (1994). Abnormality of cerebellar vermian lobules VI and VII in patients with infantile autism: Identification of hypoplastic and hyperplastic subgroups with MR imaging. *American Journal of Roentgenology, 162,* 123–130.

Courchesne, E., Townsend, J., Akshoomoff, N. A., Saitoh, O., Yeung–Courchesne, R., Lincoln, A. J., James, H. E., Haas, R. H., Schreibman, L., & Lau, L. (1994). Impairment in shifting attention in autistic and cerebellar patients. *Behavioral Neuroscience, 108,* 848–865.

Courchesne, E., Townsend, J., & Saitoh, O. (1994). The brain in infantile autism: Posterior fossa structures are abnormal [See comments]. *Neurology, 44,* 214–223.

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

Courchesne, E., Yeung–Courchesne, R., Press, G. A., Hesselink, J. R., & Jernigan, T. L. (1988). Hypoplasia of cerebellar vermal lobules VI and VII in autism. *New England Journal of Medicine, 318,* 1349–1354.

Cox, A., Klein, K., Charman, T., Baird, G., Baron–Cohen, S., Swettenham, J., Drew, A., & Wheelwright, S. (1999). Autism spectrum disorders at 20 and 42 months of age: Stability of clinical and ADI-R diagnosis. *Journal of Child Psychology and Psychiatry and Allied Disciplines, 40,* 719–732.

Creasey, H., Rumsey, J. M., Schwartz, M., Duara, R., Rapoport, J. L., & Rapoport, S. I. (1986). Brain morphometry in autistic men as measured by volumetric computed tomography. *Archives of Neurology, 43,* 669–672.

Dawson, G., & Lewy, A. (1989). Arousal, attention, and the socioemotional impairments of individuals with autism. In G. Dawson (Ed.), *Autism: Nature, diagnosis, and treatment* (pp. 49–74). New York: Guilford Press.

DiLavore, P. C., Lord, C., & Rutter, M. (1995). The Pre-Linguistic Autism Diagnostic Observation Schedule. *Journal of Autism and Developmental Disorders, 25,* 355–379.

Durston, S., Hulshoff, H. E., Casey, B. J., Giedd, J. N., Buitelaar, J. K., & van Engeland, H. (2001). Anatomical MRI of the developing brain: What have we learned? *Journal of the American Academy of Child and Adolescent Psychiatry, 40,* 1012–1020.

Egaas, B., Courchesne, E., & Saitoh, O. (1995). Reduced size of corpus callosum in autism. *Archives of Neurology, 52,* 794–801.

Elman, J. L., Bates, E. A., Johnson, M. H., Karmiloff–Smith, A., Parisi, D., & Plunkett, K. (1996). *Rethinking innateness: A connectionist perspective on development*. Cambridge, MA: Bradford/MIT Press.

Fatemi, S. H., Halt, A. R., Earle, J., Kist, D. A., Realmuto, G., Thuras, P. D., & Merz, A. (2000). Reduced Purkinje cell size in autistic cerebellum. *Biological Psychiatry, 47,* 128S.

Fatemi, S. H., Halt, A. R., Stary, J. M., Realmuto, G. M., & Jalali–Mousavi, M. (2001). Reduction in anti-apoptotic protein Bcl-2 in autistic cerebellum. *NeuroReport, 12,* 929–933.

Fehlow, P., Bernstein, K., Tennstedt, A., & Walther, F. (1993). Early infantile autism and excessive aerophagy with symptomatic megacolon and ileus in a case of Ehlers–Danlos syndrome [In German]. *Padiatrie und Grenzgebiete, 31,* 259–267.

Filipek, P. A. (1995). Quantitative magnetic resonance imaging in autism: The cerebellar vermis. *Current Opinion in Neurology, 8,* 134–138.

Gaffney, G. R., Kuperman, S., Tsai, L. Y., & Minchin, S. (1989). Forebrain structure in infantile autism. *Journal of the American Academy of Child and Adolescent Psychiatry, 28*(4).

Gaffney, G. R., Tsai, L. Y., Kuperman, S., & Minchin, S. (1987). Cerebellar structure in autism. *American Journal of Diseases of Children, 141,* 1330–1332.

Garber, H. J., & Ritvo, E. R. (1992). Magnetic resonance imaging of the posterior fossa in autistic adults [See comments]. *American Journal of Psychiatry, 149,* 245–247.

Garber, H. J., Ritvo, E. R., Chiu, L. C., Griswold, V. J., Kashanian, A., Freeman, B. J., & Oldendorf, W. H. (1989). A magnetic resonance imaging study of autism: Normal fourth ventricle size and absence of pathology. *American Journal of Psychiatry, 146,* 532–534.

Gelehrter, T. D., & Collins, F. S. (1990). *Principles of medical genetics*. Baltimore, MD: Williams & Wilkins.

Hardan, A. Y., Minshew, N. J., Harenski, K., & Keshavan, M. S. (2001). Posterior fossa magnetic resonance imaging in autism. *Journal of the American Academy of Child and Adolescent Psychiatry, 40,* 666–672.

Harris, N. S., Courchesne, E., Townsend, J., Carper, R. A., & Lord, C. (1999). Neuroanatomic contributions to slowed orienting of attention in children with autism. *Cognitive Brain Research, 8,* 61–71.

Hashimoto, T., Tayama, M., Murakawa, K., Yoshimoto, T., Miyazaki, M., Harada, M., & Kuroda, Y. (1995). Development of the brainstem and cerebellum in autistic patients. *Journal of Autism and Developmental Disorders, 25,* 1–18.

Haznedar, M. M., Buchsbaum, M. S., Wei, T.–C., Hof, P. R., Cartwright, C., Bienstock, C. A., & Hollander, E. (2000). Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonace imaging. *American Journal of Psychiatry, 157,* 1994–2001.

Holttum, J. R., Minshew, N. J., Sanders, R. S., & Phillips, N. E. (1992). Magnetic resonance imaging of the posterior fossa in autism. *Biological Psychiatry, 32,* 1091–1101.

Howard, M. (2000). Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. *Neuroreport, 11,* 2931–2935.

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology, 387,* 167–178.

Jacobson, R., Le Couteur, A., Howlin, P., & Rutter, M. (1988). Selective subcortical abnormalities in autism. *Psychological Medicine, 18,* 39–48.

Jessell, T. M., & Sanes, J. R. (2000). Development. The decade of the developing brain. *Current Opinion in Neurobiology, 10,* 599–611.

Kates, W. R., Mostofsky, S. H., Zimmerman, A. W., Mazzocco, M. M., Landa, R., Warsofsky, I. S., Kaufmann, W. E., & Reiss, A. L. (1998). Neuroanatomical and neurocognitive differences in a pair of monozygous twins discordant for strictly defined autism. *Annals of Neurology, 43,* 782–791.

Kemper, T., & Bauman, M. (1998). Neuropathology of infantile autism. *Journal of Neuropathology and Experimental Neurology, 57,* 645–652.

Killackey, H. P. (1990). Neocortical expansion: An attempt toward relating phylogeny and onotogeny. *Journal of Cognitive Neuroscience, 2,* 1–17.

Kleiman, M. D., Neff, S., & Rosman, N. P. (1992). The brain in infantile autism: Are posterior fossa structures abnormal? *Neurology, 42,* 753–760.

Klin, A., & Volkmar, F. R. (1997). Asperger syndrome. In D. J. Cohen & F. R. Volkmar (Eds.), *Handbook of autism and pervasive developmental disorders* (2nd ed., pp. 94–122). New York: Wiley.

Kolb, B., & Whishaw, I. (1989). Plasticity in the neocortex: mechanisms underlying recovery from early brain damage. *Progress in Neurobiology, 32,* 235–276.

Koul, O. (2001). *Myelin and autism.* Paper presented at the International Meeting for Autism Research, San Diego, CA.

Levitt, J., Blanton, R., Capetillo–Cunliffe, L., Guthrie, D., Toga, A., & McCracken, J. (1999). Cerebellar

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

vermis lobules VIII–X in autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 23,* 625–633.

Lewin, G. R., & Barde, Y. A. (1996). The physiology of neurotrophins. *Annual Review of Neuroscience, 19,* 289–317.

Lord, C. (1995). Follow-up of two-year-olds referred for possible autism. *Journal of Child Psychology and Psychiatry, 36,* 1365–1382.

Lord, C., & Risi, S. (2000). Diagnosis of autism spectrum disorders in young children. In A. M. Wetherby & B. M. Prizant (Eds.), *Autism spectrum disorders: A transactional developmental perspective* (pp. 11–30). Baltimore, MD: Paul H. Brookes Publishing.

Lord, C., Risi, S., Lambrecht, L., Cook, Jr., E., Leventhal, B., DiLavore, P., Pickles, A., & Rutter, M. (2000). The Autism Diagnostic Observation Schedule—Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders, 30,* 205–223.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview–Revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24,* 659–685.

Lovaas, O. I., Koegel, R. L., & Schreibman, L. (1979). Stimulus overselectivity in autism: A review of research. *Psychological Bulletin, 86,* 1236–1254.

Makeig, S., Westerfield, M., Jung, T.–P., Covington, J., Townsend, J., Sejnowski, T. J., & Courchesne, E. (1999). Functionally independent components of the late positive event-related potential during visual spatial attention. *Journal of Neuroscience, 19,* 2665–2680.

Manes, F., Piven, J., Vrancic, D., Nanclares, V., Plebst, C., & Starkstein, S. E. (1999). An MRI study of the corpus callosum and cerebellum in mentally retarded autistic individuals. *Journal of Neuropsychiatry and Clinical Neurosciences, 11,* 470–474.

Miles, J. H., & Hillman, R. E. (2000). Value of a clinical morphology examination in autism [See comments]. *American Journal of Medical Genetics, 91,* 245–253.

Mills, D. L., Coffey–Corina, S. A., & Neville, H. J. (1994). Variability in cerebral organization during primary language acquisition. In G. Dawson & K. W. Fischer (Eds.), *Human behavior and the developing brain* (pp. 427–455). New York: Guilford Press.

Morrison, M. E., & Mason, C. A. (1998). Granule neuron regulation of purkinje cell development: Striking a balance between neurotrophin and glutamate signaling. *Journal of Neuroscience, 18,* 3563–3573.

Mostofsky, S. H., Goldberg, M. C., Landa, R. J., & Denckla, M. B. (2000). Evidence for a deficit in procedural learning in children and adolescents with autism: implications for cerebellar contribution. *Journal of the International Neuropsychological Society, 6,* 752–759.

Müller, R.–A., & Courchesne, E. (2000). The duplicity of plasticity: A conceptual approach to the study of early lesion and developmental disorders. In M. Ernst & J. Rumsey (Eds.), *The foundation and future of functional neuroimaging in child psychiatry* (pp. 335–365). New York: Cambridge University Press.

Müller, R.–A., Pierce, K., Ambrose, J. B., Allen, G., & Courchesne, E. (2001). Atypical patterns of cerebral motor activation in autism: A functional magnetic resonance study. *Biological Psychiatry, 49,* 665–676.

Murakami, J. W., Courchesne, E., Press, G. A., Yeung–Courchesne, R., & Hesselink, J. R. (1989). Reduced cerebellar hemisphere size and its relationship to vermal hypoplasia in autism. *Archives of Neurology, 46,* 689–694.

Nelson, K. B., Grether, J. K., Croen, L. A., Dambrosia, J. M., Dickens, B. F., Jelliffe, L. L., Hansen, R. L., & Phillips, T. M. (2001). Neuropeptides and neurotrophins in neonatal blood of children with autism or mental retardation. *Annals of Neurology, 49,* 597–606.

O'Leary, D. D. (1992). Development of connectional diversity and specificity in the mammalian brain by the pruning of collateral projections. *Current Opinion in Neurobiology, 2,* 70–77.

O'Leary, D. D. M. (1989). Do cortical areas emerge from a protocortex? *Trends in Neuroscience, 12,* 400–406.

Petersen, S. E., Fox, P. T., Posner, M. I., Mintun, M., & Raichle, M. E. (1989). Positron emission tomographic studies of the processing of single words. *Journal of Cognitive Neuroscience, 1,* 153–170.

Pierce, K., & Courchesne, E. (2001). Restricted interests and stereotyped behaviors in autism: The role of the cerebellum. *Biological Psychiatry, 49,* 655–664.

Pierce, K., Glad, K. S., & Schreibman, L. (1997). Social perception in children with autism: An attentional deficit? *Journal of Autism and Developmental Disorders, 27,* 265–282.

Pierce, K., Müller, R–A., Ambrose, J., Allen, G., & Courchesne, E. (2001). Face processing occurs outside the fusiform "face area" in autism: Evidence from functional MRI. *Brain, 124,* 2059–2073.

Piven, J., & Arndt, S. (1995). The cerebellum and autism. *Neurology, 45,* 398–402.

Piven, J., Arndt, S., Bailey, J., & Andreasen, N. (1996). Regional brain enlargement in Autism: A magnetic resonance imaging study. *Journal of the American Academy of Child and Adolescent Psychiatry, 35,* 530–536.

Piven, J., Bailey, J., Ranson, B. J., & Arndt, S. (1997). An MRI study of the corpus callosum in autism. *American Journal of Psychiatry, 154,* 1051–1055.

Piven, J., Bailey, J., Ranson, B. J., & Arndt, S. (1998). No difference in hippocampus volume detected on magnetic resonance imaging in autistic individuals. *Journal of Autism and Developmental Disorders, 28,* 105–110.

Piven, J., Nehme, E., Simon, J., Barta, P., Pearlson, G., & Folstein, S. E. (1992). Magnetic resonance imaging in autism: Measurement of the cerebellum, pons, and fourth ventricle. *Biological Psychiatry, 31,* 491–504.

Piven, J., Saliba, K., Bailey, J., & Arndt, S. (1997). An MRI study of autism: The cerebellum revisited. *Neurology, 49,* 546–551.

Piven, J., Saliba, K., Bailey, J., & Arndt, S. (1999). An MRI study of autism: The cerebellum revisited [Letter to editor]. *Neurology, 52,* 1106–1107.

Quartz, S. R., & Sejnowski, T. J. (1998). The neural basis of cognitive development: A constructivist manifesto. *Behavioral and Brain Sciences, 20,* 537–596.

Rakic, P. (Ed.). (1995). *Corticogenesis in human and nonhuman primates*. Cambridge, MA: MIT Press.

Rao, S. M., Bobholz, J. A., Hammeke, T. A., Rosen, A. C., Woodley, S. J., Cunningham, J. M., Cox, R. W., Stein, E. A., & Binder, J. R. (1997). Functional MRI evidence for subcortical participation in conceptual reasoning skills. *Neuroreport, 8,* 1987–1993.

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press

Raymond, G. V., Bauman, M. L., & Kemper, T. L. (1996). Hippocampus in autism: A Golgi analysis. *Acta Neuropathologica, 91,* 117–119.

Ritvo, E. R., Freeman, B. J., Scheibel, A. B., Duong, T., Robinson, H., Guthrie, D., & Ritvo, A. (1986). Lower Purkinje cell counts in the cerebella of four autistic subjects: Initial findings of the UCLA–NSAC Autopsy Research Report. *American Journal of Psychiatry, 143,* 862–866.

Rodier, P. M., Ingram, J. L., Tisdale, B., Nelson, S., & Romano, J. (1996). Embryological origin for autism: Developmental anomalies of the cranial nerve motor nuclei. *Journal of Comparative Neurology, 370,* 247–261.

Rosenbloom, S., Campbell, M., George, A. E., Kricheff, II, Taleporos, E., Anderson, L., Reuben, R. N., & Korein, J. (1984). High resolution CT scanning in infantile autism: A quantitative approach. *Journal of the American Academy of Child Psychiatry, 23,* 72–77.

Saitoh, O., Karns, C., & Courchesne, E. (2001). Development of the hippocampal formation from 2 to 42 years: MRI evidence of smaller area dentata in autism. *Brain, 124,* 1317–1324.

Schopler, E., Reichler, R. J., DeVellis, R. F., & Daly, K. (1980). Toward objective classification of childhood autism: Childhood Autism Rating Scale CARS. *Journal of Autism and Developmental Disorders, 10,* 91–103.

Schreibman, L., & Lovaas, O. I. (1973). Overselective response to social stimuli by autistic children. *Journal of Abnormal Child Psychology, 1,* 152–168.

Schultz, R. T., Gauthier, I., Klin, A., Fulbright, R. K., Anderson, A. W., Volkmar, F., Skudlarski, P., Lacadie, C., Cohen, D. J., & Gore, J. C. (2000). Abnormal ventral temporal cortical activity among individuals with autism and Asperger syndrome during face discrimination. *Archives of General Psychiatry, 57*.

Stiles, J. (1998). The effects of early focal brain injury on lateralization of cognitive function. *Current Directions in Psychological Science, 7,* 21–26.

Stone, W. L., Lee, E. B., Ashford, L., Brissie, J., Hepburn, S. L., Coonrod, E. E., & Weiss, B. H. (1999). Can autism be diagnosed accurately in children under 3 years? *Journal of Child Psychology and Psychiatry and Allied Disciplines, 40,* 219–226.

Townsend, J., & Courchesne, E. (1994). Parietal damage and narrow "spotlight" spatial attention. *Journal of Cognitive Neuroscience, 6,* 220–232.

Townsend, J., Courchesne, E., & Egaas, B. (1996). Slowed orienting of covert visual–spatial attention in autism: Specific deficits associated with cerebellar and parietal abnormality. *Development and Psychopathology, 8,* 563–584.

Townsend, J., Courchesne, E., Singer–Harris, N., Covington, J., Westerfield, M., Lyden, P., Lowry, T. P., & Press, G. A. (1999). Spatial attention deficits in patients with acquired or developmental cerebellar abnormality. *Journal of Neuroscience, 19,* 5632–5642.

Townsend, J., Harris, N. S., & Courchesne, E. (1996). Visual attention abnormalities in autism: Delayed orienting to location. *Journal of the International Neuropsychological Society, 2,* 541–550.

Townsend, J., Westerfield, M., Leaver, E., Makeig, S., Jung, T.-P., Pierce, K., & Courchesne, E. (2001). Event-related brain response abnormalities in autism: Evidence for impaired cerebello–frontal spatial attention networks. *Cognitive Brain Research, 11,* 127–145.

Waterhouse, L., Fein, D., & Modahl, C. (1996). Neurofunctional mechanisms in autism. *Pychological Review, 103,* 457–489.

Weimann, J., Zhang, Y., Levin, M., Devine, W., Brûlet, P., & McConnell, S. (1999). Cortical neurons require Otx1 for the refinement of exuberant axonal projections to subcortical targets. *Neuron, 24,* 819–831.

Williams, R. S., Hauser, S. L., Purpura, D. P., DeLong, G. R., & Swisher, C. N. (1980). Autism and mental retardation: Neuropathologic studies performed in four retarded persons with autistic behavior. *Archives of Neurology, 37,* 749–753.

Yeung–Courchesne, R., & Courchesne, E. (1997). From impasse to insight in autism research: From behavioral symptoms to biological explanations. *Development and Psychopathology, 9,* 389–419.

Zilbovicius, M., Barthelemy, C., Belin, P., Remy, P., Poline, J.-B., Mangin, J.-F., Syrota, A., & Samson, Y. (1998). Bitemporal hypoperfusion in childhood autism. *Neuroimage, 7,* S503.

Zilbovicius, M., Garreau, B., Samson, Y., Remy, P., Barthelemy, C., Syrota, A., & Lelord, G. (1995). Delayed maturation of the frontal cortex in childhood autism. *American Journal of Psychiatry, 152,* 248–252.

https://doi.org/10.1017/S0954579402003115 Published online by Cambridge University Press