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Brain structural abnormalities in young children with autism spectrum disorder

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Abstract—Objective: To explore the specific gross neuroanatomic substrates of this brain developmental disorder, the authors examine brain morphometric features in a large sample of carefully diagnosed 3- to 4-year-old children with autism spectrum disorder (ASD) compared with age-matched control groups of typically developing (TD) children and developmentally delayed (DD) children. **Methods:** Volumes of the cerebrum, cerebellum, amygdala, and hippocampus were measured from three-dimensional coronal MR images acquired from 45 children with ASD, 26 TD children, and 14 DD children. The volumes were analyzed with respect to age, sex, volume of the cerebrum, and clinical status. **Results:** Children with ASD were found to have significantly increased cerebral volumes compared with TD and DD children. Cerebellar volume for the ASD group was increased in comparison with the TD group, but this increase was proportional to overall increases in cerebral volume. The DD group had smaller cerebellar volumes compared with both of the other groups. Measurements of amygdalae and hippocampi in this group of young children with ASD revealed enlargement bilaterally that was proportional to overall increases in total cerebral volume. There were similar findings of cerebral enlargement for both girls and boys with ASD. For subregion analyses, structural abnormalities were observed primarily in boys, although this may reflect low statistical power issues because of the small sample (seven girls with ASD) studied. Among the ASD group, structural findings were independent of nonverbal IQ. In a subgroup of children with ASD with strictly defined autism, amygdalar enlargement was in excess of increased cerebral volume. **Conclusions:** These structural findings suggest abnormal brain developmental processes early in the clinical course of autism. Research currently is underway to better elucidate mechanisms underlying these structural abnormalities and their longitudinal progression.

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Autism is a clinically defined behavioral syndrome that initially manifests in early childhood and is thought to reflect underlying neurodevelopmental abnormalities.^{1,2} Core symptoms of autism include abnormal or unreciprocated interpersonal and emotional interactions, disordered language and communication, and repetitive and stereotypic behavior.³ Autism is not associated with specific physical stigmata and is variably associated with mental retardation and seizures. Although the neurobiologic mechanisms underlying autism remain largely unknown, certain brain regions, including the limbic system, particularly the hippocampus, amygdala, and cerebellum, have been implicated in the clinical expression and pathophysiologic mechanism of the disorder.^{4–12} Neuroimaging and postmortem studies, mostly of adolescents and adults, have found variable evidence of structural abnormalities in those regions of the brain.^{6,9–25} Increased cerebral volume or brain weight, although not consistently found

across all studies, also has been associated with autism.^{13,22–31} Limited evidence from a small series of postmortem examinations, CT studies, and a recent MRI study that evaluated children cross-sectionally between ages 2 and 16, suggests that findings of increased brain weight or brain volume may be age related and reflect accelerated developmental processes in younger children with autism.^{22,26,31} However, evidence of brain enlargement also has been found when studying older populations of autistic individuals.^{25,27–30}

Currently, few imaging studies have evaluated young children with autism. We characterized brain structural abnormalities early in the clinical course of autism as part of an ongoing longitudinal study of brain development. For this report, MRI was used to measure cerebral, cerebellar, hippocampal, and amygdalar volumes in 3- to 4-year-old children with idiopathic autistic spectrum disorder (ASD) (i.e., unrelated to known genetic abnormality or environ-

See also pages 158 and 175

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mental insult) compared with similarly aged children with idiopathic developmental delays (DD) and children with typical development (TD).

Methods. Participants. Three groups of children participated in the study: 1) 45 children with ASD (7 girls, 38 boys, mean age 47.4 ± 4.2 SD; range 38 to 54 months), including 29 children with autistic disorder (AD) (3 girls, 26 boys, mean age 46.9 ± 4.3 SD; range 38 to 54 months) and 16 children with pervasive developmental disorder not otherwise specified (PDD-NOS) (4 girls, 12 boys, mean age 48.2 ± 4.0 SD; range 42 to 54 months), as defined later; 2) 26 children with TD (8 girls, 18 boys, mean age 47.5 ± 6.2 SD; range 36 to 56 months); and 3) 14 children with DD (8 girls, 6 boys, mean age 47.5 ± 5.6 ; range 40 to 58 months). Of the children studied, two children with ASD and one TD child had MRI scans with poorly defined subregions that could not be accurately measured.

Children in the ASD and DD groups exhibited significant developmental delay as demonstrated on standardized tests of intellectual and adaptive abilities, and their developmental delay could not be attributed to known genetic syndrome (e.g., fragile X syndrome, Down syndrome), prenatal or postnatal brain trauma, or a defined disease. Each of these children was evaluated at a multidisciplinary research center where the ASD and DD children were diagnosed or had their original diagnosis confirmed. Those children were evaluated using the Mullen Scales of Early Learning³² and the Vineland Adaptive Behavior Scales,³³ both well-validated, normalized measures of development to confirm current degree of developmental delay. ASD and DD children were evenly matched on the Mullen Composite Age Equivalent score³² (25.9 ± 9.2 months vs 25.5 ± 8.1 months). For all three groups (ASD, TD, and DD), children having significant motor or sensory impairment (e.g., blindness, deafness), major physical abnormalities, seizures, history of serious head injury, identifiable neurologic disorder, prenatal or perinatal difficulties, metal implants such as prostheses, or those taking psychoactive medications on a regular basis were excluded. Children studied at the University of Washington (UW) were recruited from local parent advocacy groups, preschools, the Department of Developmental Disabilities, clinics and hospitals in the greater Seattle area, and the University of Washington Infant and Child Subject Pool. The TD group included 13 children studied at the UW and 13 studied at the NIH. Children in the UW TD group were excluded if they scored 1 SD below or above normal on the Vineland Adaptive Behavior Scales³³; the latter criterion was used to avoid having an atypically high IQ and thus a nonrepresentative sample, which often is associated with university-based studies. Moreover, for the UW TD sample, there were no parental reports of language, social, motor, or cognitive delay; speech therapy; emotional or psychiatric disturbances; or special services for learning problems. Children comprising the NIH TD sample were recruited from the community in response to local newspaper advertisements and postings. Those children underwent 1) an evaluation process involving initial telephone screening to rule out use of medications, familial history of psychiatric illness, or special service requirements in school; and 2) an on-site physical, structured

psychiatric evaluation (J.N.G.) and neuropsychological examination at the NIH to exclude children with psychiatric illness, neurologic disorders, or DD. The neuropsychological examination included subtests from the Wechsler Intelligence Scale for Children–Revised³⁴ on which the TD children scored average or better. Further details of the screening, inclusion, and exclusion criteria for the NIH TD children are presented elsewhere.³⁵ Written informed consent from the parent or guardian, approved by the UW Internal Review Board or the NIH Internal Review Board, was obtained for each child participating in the study.

Children in the ASD group were administered a diagnostic battery consisting of the Autism Diagnostic Interview–Revised (ADI-R),³⁶ the Autism Diagnostic Observation Schedule–Generic (ADOS-G),^{37,38} and clinical assessment by a highly trained clinician. This assessment evaluated for symptoms of AD and PDD-NOS listed in the *Diagnostic and Statistical Manual of Mental Disorders, 4th edition* (DSM-IV).³ The ASD group was further divided into subgroups of AD and PDD-NOS based on the extent of symptom manifestation, integrating findings from the clinical assessment and two diagnostic instruments, using criteria applied in a consistent manner.³⁸ Specifically, children in the AD group met criteria for autism on the ADOS-G and ADI-R (or within two points of criteria) and by DSM-IV–based clinical assessment. The PDD-NOS diagnosis was made if the child met criteria for autism on the ADI-R but was classified as PDD-NOS on the ADOS-G and by DSM-IV–based clinical assessment. The ADOS-G was administered to the DD and TD children at the UW. These children did not meet criteria for either AD or PDD-NOS based on the ADOS-G or on a clinical judgment using DSM-IV criteria, nor did they show elevated symptoms of autism on these measures.

MRI scans. Children with ASD and DD underwent imaging during continuous IV infusion of propofol at 180 to 220 $\mu\text{g}/\text{kg}$ per minute. TD children, both at the UW and at NIH, were scanned late at night while asleep. For the UW TD sample, children were sedated on an optional basis with parentally administered diphenhydramine hydrochloride ($n = 8$, 25 mg PO) if the child previously had experienced sedation when given this agent. No sedation was used for the NIH TD sample. Imaging studies at the UW were performed on a 1.5-T GE Signa Scanner. A three-dimensional SPGR imaging sequence (repetition time [TR] = 33 ms, echo time [TE] = minimum, flip angle = 30, 22-cm field of view [FOV], and 256×256 matrix) acquired in the coronal plane was used for volume determinations. During acquisition, a 3-mm slice thickness was reduced to 1.5 mm through zero filling in the third-phase encoding direction to improve resolution (i.e., the effective partition thickness) without loss in signal-to-noise ratio. Scanning of TD children at the NIH used an identical GE scanner and similar acquisition parameters with the exception that coronal slice thickness was 2 mm (no zero filling) at a 24-cm FOV.

Structural measurements. Volumetric measurements of total cerebrum, cerebellum, bilateral hippocampi, and amygdalae were performed by a single rater blinded to diagnosis (B.F.S.). Volumetric measurements (in cubic centimeters) were determined using MEASURE, a semiautomated imaging analysis program developed at Johns Hopkins University,³⁹ which allows simultaneous data vi-

Table 1 Raw group means (\pm SD) for brain regions in children with autism spectrum disorder (ASD), typical development (TD), and delayed development (DD)

Brain region	ASD,* n = 45	TD,* n = 26	DD,* n = 14
Cerebrum	1191.87 (94.71)	1085.87 (109.20)	1059.23 (128.97)
Cerebellum	134.22 (13.92)	124.75 (16.70)	96.40 (33.78)
Right hippocampus	2.67 (0.32)	2.46 (0.30)	2.06 (0.49)
Left hippocampus	2.74 (0.32)	2.51 (0.32)	2.07 (0.49)
Right amygdala	1.19 (0.26)	1.02 (0.23)	1.02 (0.22)
Left amygdala	1.25 (0.23)	1.10 (0.20)	1.06 (0.26)

* Values represent volumes measured in milliliters.

sualization and interaction within multiple planes (coronal, axial, sagittal). Total cerebral volume and cerebellar volume were measured using a three-dimensional stereotaxic grid consisting of $17 \times 17 \times 17$ grid points. The size of the grid was determined by an output of 200 points or more within the region of interest.⁴⁰ All points consisting of gray and white brain tissue were included, whereas points lying within CSF or non-brain tissue were excluded. Cerebral volume included the basal ganglia and corpus callosum and excluded the ventricles, brainstem, and cerebellum. At the level of the cerebellar peduncles, the cerebellum and brainstem were separated from the cerebrum by drawing a straight line from the most anterolateral point of the fourth ventricle to the "notch" created by the junction of the brainstem and cerebellum.

The blinded rater used rules and detailed anatomy previously described to manually trace hippocampi.⁴¹ Tracing of the right and left hippocampi was performed in the coronal view starting at the most posterior slice where hippocampal gray matter could be unambiguously discerned. The choroid fissure and the inferior horn of the lateral ventricle served as the superior border of the hippocampi. The lateral border for each side was defined by the inferior temporal horn of the lateral ventricle or temporal stem white matter. The inferior border for each side was defined by the parahippocampal gyrus. The mesial boundary followed the angle where the hippocampus curved inferiorly and medially into the parahippocampal gyrus; any tissue lateral and superior to the parahippocampal gyrus was included, and any tissue medial or inferior was excluded. Both the alveus and subiculum were included in volume measurements, whereas the uncus and any structures medial to the parahippocampal gyrus were omitted. Sagittal and axial views were used to confirm boundary placement and anterior–posterior definition.

Coronal images were reformatted into the axial plane, resulting in slices that were 0.94-mm or 0.86-mm thick (depending on FOV) for tracing the amygdalae. The landmarks used to define amygdalar boundaries have been described previously.⁴¹ Briefly, the superior border was set at the level of the tubera where the optic nerve was clearly separated from the mamillary body. The lateral border was defined by a vertical line from the extreme edge of the most medial white matter protruding into the amygdaline gray matter. The medial boundary was defined by the uncus. The posterior border was set at the temporal horn of the lateral ventricles in superior slices and at the head of the hippocampi for inferior slices. Sagittal and coronal views were consulted to confirm boundary placement, and

any tissue anterior to the anterior commissure was removed. In addition, any tissue medial to the uncus notch in the coronal plane was excluded.

Six-month intra-rater reliability (B.F.S.) using intraclass correlations based on five child scans for volumetric measurements was 0.92 for left hippocampus (2.63 ± 0.25 vs 2.62 ± 0.15) and 0.92 for left amygdala (1.27 ± 0.33 vs 1.21 ± 0.31). Interrater reliability (B.F.S. vs E.H.A.) for volumetric measurements on five adult scans was 0.83 for left hippocampus (2.78 ± 0.50 vs 2.94 ± 0.55) and 0.99 for left amygdala (1.81 ± 0.32 vs 1.83 ± 0.30).

Statistical methods. Statistical analysis was performed using SPSS statistical software (Chicago, IL). All group analyses were performed using analysis of covariance (ANCOVA), with covariance for age and sex. Additional statistical analyses using ANCOVA used covariance for cerebral volume, as well as age and sex, in evaluating subregion differences between groups. All values are presented with standard deviations (\pm SD) and reflect means.

Results. There were no significant age differences between diagnostic groups [$F(2,82) = 0.002$]. Group differences in sex were demonstrated using Pearson χ^2 analysis ($\chi^2 = 9.62$, $df = 2$, $p < 0.01$), a result of more girls in the DD group (58%) relative to the ASD (16%) ($\chi^2 = 9.74$, $df = 1$, $p = 0.002$) and TD (30%) ($\chi^2 = 2.64$, $df = 1$, $p = 0.10$) groups. There were no significant sex differences between ASD and TD groups ($\chi^2 = 2.29$, $df = 1$). In addition, sex distribution was similar between the AD and PDD-NOS subgroups ($\chi^2 = 1.69$, $df = 1$). However, because both age and sex factors are known to affect brain size, volumetric data were analyzed with covariance for age and sex to minimize any influence of these demographic variables on volumetric comparisons between groups. Because comparisons of cerebral volume and discrete subregional structures were not different between UW and NIH TD samples ($F < 1.86$ for all, $p > 0.2$ for all), the TD samples were combined for all further analyses.

Volume measurements for the three primary diagnostic groups are shown in table 1. In table 2, statistical analyses of volume relationships between groups using ANCOVA, with covariance for age and sex, are summarized. Moreover, because various approaches have been used to scale for brain volume influences on subregion comparisons,⁴² additional analyses including cerebral volume as a covariate for subregion analyses are similarly detailed in table 2. The ASD group demonstrated significant cerebral enlargement in comparison with both TD (9.76%) and DD (12.52%) groups. In contrast, no significant differences be-

Table 2 Between diagnostic group structural comparisons using covariate analyses (ANCOVA) for brain regions in children with autism spectrum disorder (ASD), typical development (TD), and delayed development (DD)

Brain region	Group comparisons	ANCOVA (age and sex)	ANCOVA (age, sex, and cerebral volume)
Cerebrum	ASD vs TD	$F_{(1,67)} = 16.44, p < 0.001$	
	ASD vs DD	$F_{(1,55)} = 8.82, p = 0.004$	
	TD vs DD	$F_{(1,36)} = 0.04, p = \text{NS}$	
Cerebellum	ASD vs TD	$F_{(1,67)} = 5.07, p = 0.03$	$F_{(1,66)} = 0.01, p = \text{NS}$
	ASD vs DD	$F_{(1,55)} = 24.22, p < 0.001$	$F_{(1,54)} = 13.04, p = 0.001$
	TD vs DD	$F_{(1,36)} = 9.37, p = 0.004$	$F_{(1,35)} = 25.17, p < 0.001$
Right hippocampus	ASD vs TD	$F_{(1,66)} = 5.70, p = 0.02$	$F_{(1,65)} = 2.19, p = \text{NS}$
	ASD vs DD	$F_{(1,55)} = 18.30, p = 0.001$	$F_{(1,54)} = 9.33, p = 0.003$
	TD vs DD	$F_{(1,35)} = 7.27, p = 0.01$	$F_{(1,34)} = 7.96, p = 0.008$
Left hippocampus	ASD vs TD	$F_{(1,66)} = 6.84, p = 0.01$	$F_{(1,65)} = 2.67, p = \text{NS}$
	ASD vs DD	$F_{(1,55)} = 22.68, p = 0.001$	$F_{(1,54)} = 13.25, p = 0.001$
	TD vs DD	$F_{(1,35)} = 8.28, p = 0.007$	$F_{(1,34)} = 8.88, p = 0.005$
Right amygdala	ASD vs TD	$F_{(1,65)} = 8.52, p = 0.005$	$F_{(1,64)} = 1.81, p = \text{NS}$
	ASD vs DD	$F_{(1,54)} = 2.44, p = 0.124$	$F_{(1,53)} = 0.07, p = \text{NS}$
	TD vs DD	$F_{(1,35)} = 0.04, p = \text{NS}$	$F_{(1,34)} = 0.02, p = \text{NS}$
Left amygdala	ASD vs TD	$F_{(1,65)} = 6.49, p = 0.013$	$F_{(1,64)} = 1.55, p = \text{NS}$
	ASD vs DD	$F_{(1,54)} = 3.23, p = 0.078$	$F_{(1,53)} = 0.77, p = \text{NS}$
	TD vs DD	$F_{(1,35)} = 0.16, p = \text{NS}$	$F_{(1,34)} = 0.12, p = \text{NS}$

tween cerebral volume in the TD and DD groups was observed. Volume relationships between groups (ASD, TD, and DD) for cerebrum and brain subregions, normalized to the TD sample, are shown in figure 1.

Similar to cerebrum, significant cerebellar enlargement in the ASD was observed group in comparison with the TD group (7.59%), whereas the DD group exhibited significantly reduced cerebellar volume compared with both ASD (-22.73%) and TD (-28.18%) groups. With the additional covariate of cerebral volume, cerebellar volume differences between ASD and TD groups did not remain significant. However, cerebellar volume corrected for brain size in the

DD group remained significantly decreased compared with adjusted cerebellar measures in the ASD and TD samples.

For medial temporal structures, both right and left hippocampal volumes in the ASD group were significantly increased compared with the TD group (8.54% on the right side and 9.16% on the left side), whereas the DD group demonstrated significantly reduced right and left hippocampal volumes in comparison with the ASD group (-29.61% on the right side and -32.37% on the left side) and TD group (-16.67% on the right side and -17.55% on the left side). When cerebral volume was included as an additional covariate, hippocampal volume differences no

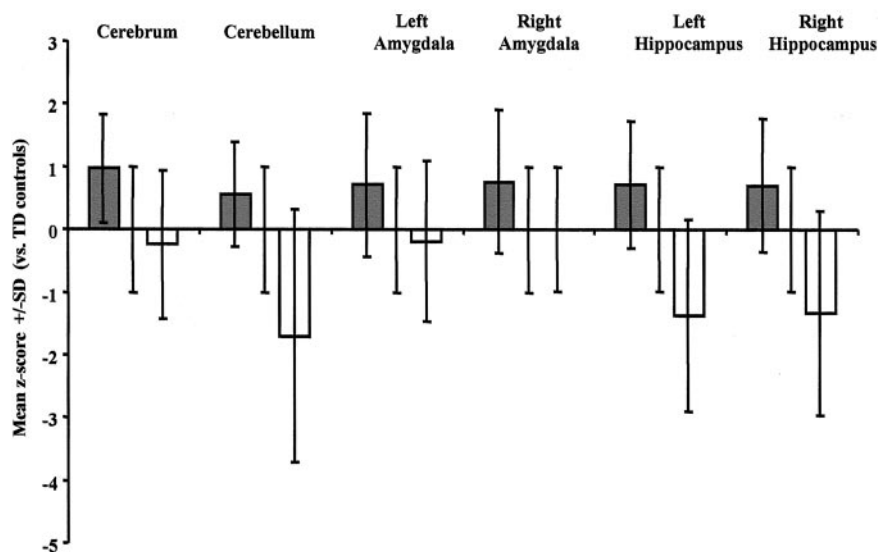


Figure 1. Volume relationships (\pm SD) normalized to the typically developing (TD) sample for children with autism spectrum disorder (gray columns), TD children (mean = 0, SD shown), and developmentally delayed children (white columns).

Table 3 Uncorrected means (\pm SD) by brain region for boys with autism spectrum disorder (ASD) and typical development (TD)

Brain region	ASD,* n = 38	TD,* n = 18	ANCOVA (covaried by age)	ANCOVA (covaried by age and cerebral volume)
Cerebrum	1202.30 (95.85)	1098.42 (117.07)	$F_{(1,53)} = 11.53, p = 0.001$	
Cerebellum	135.58 (14.40)	125.98 (18.94)	$F_{(1,53)} = 3.98, p = 0.05$	$F_{(1,52)} = 0.01, p = \text{NS}$
Right hippocampus	2.70 (0.33)	2.47 (0.31)	$F_{(1,52)} = 5.31, p = 0.025$	$F_{(1,51)} = 2.34, p = \text{NS}$
Left hippocampus	2.75 (0.34)	2.51 (0.30)	$F_{(1,52)} = 5.77, p = 0.02$	$F_{(1,51)} = 3.05, p = 0.09$
Right amygdala	1.20 (0.25)	0.94 (0.19)	$F_{(1,51)} = 13.20, p = 0.001$	$F_{(1,50)} = 5.26, p = 0.03$
Left amygdala	1.25 (0.23)	1.08 (0.20)	$F_{(1,51)} = 7.44, p = 0.009$	$F_{(1,50)} = 2.53, p = 0.12$

* Values represent volumes measured in milliliters.

longer attained significance for either side between ASD and TD groups, whereas the DD group exhibited persistent hippocampal reduction for both sides in comparison with ASD and TD groups.

The ASD group demonstrated significantly increased right and left amygdalar volumes in comparison with the TD sample (16.67% on the right side and 13.64% on the left side). A similar pattern was observed between the ASD and DD control group, with trend or significantly increased amygdalar volumes (16.67% on the right side and 17.53% on the left side). No amygdalar volume differences were demonstrated between the TD and DD groups. No amygdalar volume differences remained significant between the ASD group in comparison with either TD or DD groups when ANCOVA additionally used covariance for cerebral volume.

Although statistical analyses of brain volumetric data included covariance for the effects of sex, autism is a disorder predominately manifested in boys. To explore whether there were sex differences in brain abnormalities observed in ASD, we performed ANCOVA analyses stratifying the ASD and TD samples by sex. Descriptive data and statistical analyses by sex are summarized in tables 3 and 4. Findings indicate similar enlargement of cerebral volume across sex in the ASD children. Cerebellar size also was enlarged in ASD boys of proportionate magnitude to cerebrum. In contrast, a nonsignificant pattern of cerebellar enlargement was observed in girls with ASD. A less clearcut pattern of volumetric relationships by sex was observed for the hippocampi and amygdalae. Boys with ASD demonstrated significant bilateral enlargement for both subregions using ANCOVA, with covariance for age, and a general pattern of trend or near-trend enlargement,

scaled for cerebral volume, which reached significance in the right amygdala. The small sample of girls with ASD did not exhibit a similar pattern of subregion structural abnormalities. Volume relationships between ASD and TD groups for brain regions normalized to the combined TD sample are shown separately for boys and girls in figures 2 and 3.

Much of the literature on autism neuroimaging reports on findings from higher functioning individuals (typically defined as IQ 80 or higher); we further differentiated the entire ASD group on the basis of nonverbal IQ (NVIQ) less than 80 (n = 34) and NVIQ of 80 or higher (n = 10) for subgroup analyses. Using this stratification approach and covariance for age and sex, no significant structural differences between the high- and low-functioning ASD children were demonstrated ($F < 2.6$ for all).

Because autism is a syndrome with variable symptom expression and severity, we further stratified the ASD group into AD and PDD-NOS subgroups (as specified under Methods). Volumetric measurements and F statistics used covariance for age and sex, as well as by cerebral volume, are summarized in table 5. No differences in cerebral, cerebellar, or hippocampal volumetric measurements between the AD and PDD-NOS subgroups were demonstrated. In contrast, significant bilateral amygdalar enlargement was demonstrated in the AD subgroup relative to the PDD-NOS subjects (15.7% on the right and 13.2% on the left). Relationships between AD and PDD-NOS subgroups for brain regional volumes normalized to the TD sample are shown in figure 4. Differences in right and left amygdalar volumes between the AD and PDD-NOS subgroups remained significant when scaled for cerebral volume differences. Although not significant by χ^2 analysis,

Table 4 Uncorrected means (\pm SD) by brain region for girls with autism spectrum disorder (ASD) and typical development (TD)

Brain region	ASD,* n = 7	TD,* n = 8	ANCOVA (covaried by age)	ANCOVA (covaried by age and cerebral volume)
Cerebrum	1135.26 (68.53)	1057.62 (89.39)	$F_{(1,12)} = 9.06, p = 0.01$	
Cerebellum	126.86 (8.20)	121.98 (10.60)	$F_{(1,12)} = 2.61, p = \text{NS}$	$F_{(1,11)} = 0.06, p = \text{NS}$
Right hippocampus	2.53 (0.23)	2.43 (0.30)	$F_{(1,12)} = 0.63, p = \text{NS}$	$F_{(1,11)} = 0.06, p = \text{NS}$
Left hippocampus	2.66 (0.20)	2.49 (0.39)	$F_{(1,12)} = 2.66, p = \text{NS}$	$F_{(1,11)} = 0.01, p = \text{NS}$
Right amygdala	1.14 (0.29)	1.18 (0.22)	$F_{(1,12)} = 0.02, p = \text{NS}$	$F_{(1,11)} = 1.41, p = \text{NS}$
Left amygdala	1.20 (0.22)	1.16 (0.21)	$F_{(1,12)} = 0.16, p = \text{NS}$	$F_{(1,11)} = 0.05, p = \text{NS}$

* Values represent volumes measured in milliliters.

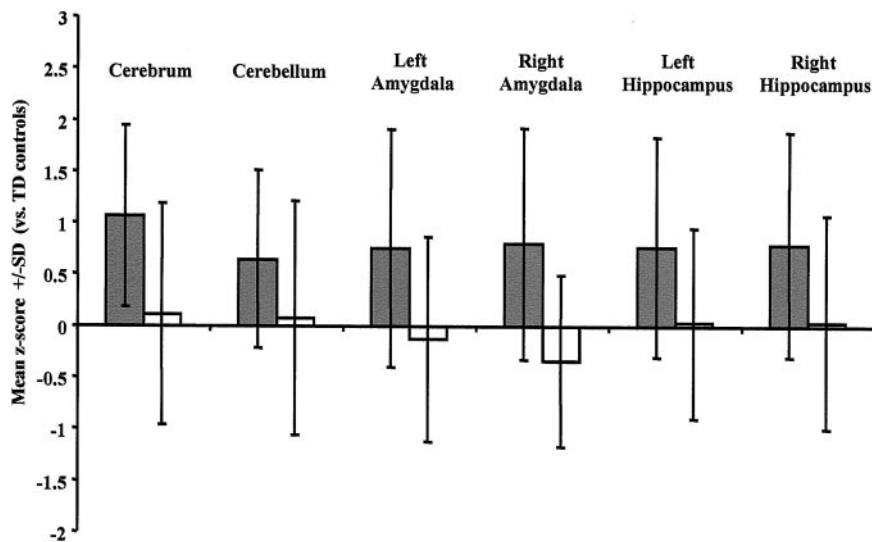


Figure 2. Volume relationships (\pm SD) normalized to the combined typically developing (TD) sample for boys with autism spectrum disorder (gray columns) and TD boys (white columns).

there were sex differences between the AD (89% boys) and PDD-NOS (75% boys) groups. To further support that volume differences were not driven by sex, additional analyses were performed between the AD and PDD-NOS boys, using covariance for age and cerebral volume, which demonstrated similar results [$F(1,33) = 5.35, p = 0.027$ for the right amygdala; $F(1,33) = 3.70, p = 0.063$ for the left amygdala].

Discussion. In this study, we report that 3- to 4-year-old children with ASD have enlarged cerebral volumes in comparison with TD and DD children. This finding of cerebral enlargement between the ASD group represents an overall 9.8% increase compared with the TD group and a 12.5% increase compared with the DD group. Although some investigators suggest a bimodal distribution for megalencephaly or macrocephaly associated with autism,^{26,27,43} we found no evidence of a bimodal distribution for cerebral volume in this large sample of children with ASD. Cerebral enlargement observed in this study was independent of IQ, which is consistent with prior observations in

adults.²⁹ We further observed that cerebral enlargement was present in both boys and girls with ASD, a relationship previously reported for young children but not adults.^{22,29} A recent cross-sectional study of children and adolescents suggests that early brain enlargement in autism decreases across the developmental course.²² However, whereas cross-sectional evaluation is useful for revealing developmental progression of a disease, the longitudinal course within individuals may vary. Because ASD children reported in this study currently are being reevaluated at 6 to 7 years of age, the progression of regional brain abnormalities can be further characterized.

We observed that cerebellar volume in the ASD children was increased compared with TD children, but this increase was proportional to overall cerebral enlargement. In comparison, the DD children had markedly smaller cerebellums, a finding that was independent of cerebral volume when compared with both of the other groups. As with results on cerebral volume, proportional enlargement of cerebellar vol-

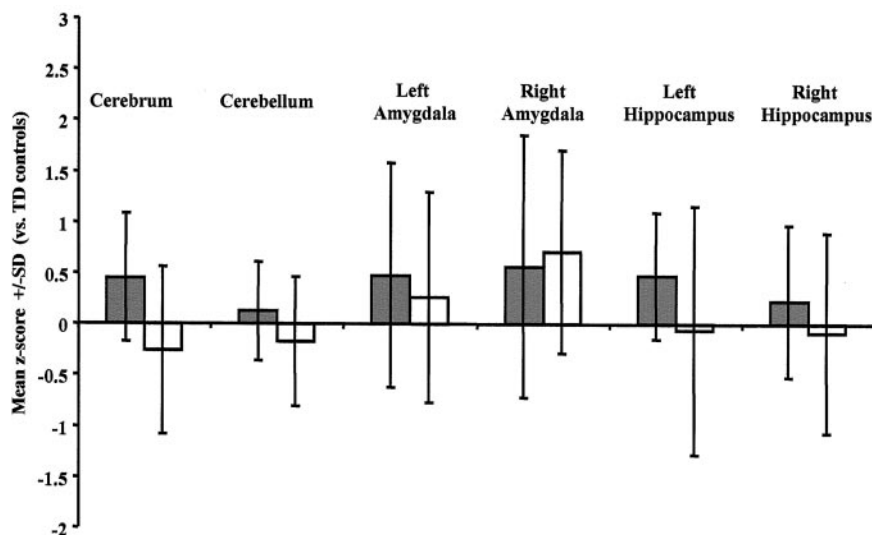


Figure 3. Volume relationships (\pm SD) normalized to the combined typically developing (TD) sample for girls with autism spectrum disorder (ASD) (gray columns) and TD girls (white columns).

Table 5 Uncorrected group means (\pm SD) and covariate analyses (ANCOVA) by brain region for autism spectrum disorder (ASD), children clinically differentiated into autism disorder (AD) and pervasive developmental disorder-not otherwise specified (PDD-NOS)

Brain region	AD,* n = 29	PDD-NOS,* n = 16	ANCOVA (covaried by sex and age)	ANCOVA (covaried by sex, age, and cerebral volume)
Cerebrum	1188.18 (83.08)	1210.88 (86.65)	$F_{(1,41)} = 0.02, p = \text{NS}$	
Cerebellum	133.13 (14.11)	136.39 (13.26)	$F_{(1,41)} = 0.46, p = \text{NS}$	$F_{(1,40)} = 0.46, p = \text{NS}$
Right hippocampus	2.69 (0.34)	2.65 (0.26)	$F_{(1,41)} = 0.04, p = \text{NS}$	$F_{(1,40)} = 0.06, p = \text{NS}$
Left hippocampus	2.77 (0.36)	2.70 (0.30)	$F_{(1,41)} = 0.21, p = \text{NS}$	$F_{(1,40)} = 0.25, p = \text{NS}$
Right amygdala	1.25 (0.24)	1.08 (0.19)	$F_{(1,40)} = 7.96, p = 0.007$	$F_{(1,39)} = 10.60, p = 0.002$
Left amygdala	1.29 (0.24)	1.14 (0.20)	$F_{(1,40)} = 5.00, p = 0.031$	$F_{(1,39)} = 5.48, p = 0.024$

* Values represent volumes measured in milliliters.

ume observed in the children with ASD also may reflect the young age range of children studied, considering that some investigators report normal or reduced cerebellar volumes in older populations.¹⁷⁻²³ Several reports have specifically investigated vermian size in autism,^{17,18} an abnormality reported to be independent of age²²; these analyses are anticipated in the future.

In the current study, we found bilateral enlargement both of the amygdalae and hippocampi in children with ASD in comparison with the TD children; the DD children demonstrated reduced hippocampal volumes bilaterally compared with both of the other groups. When cerebral volume was used as an additional covariate, any apparent subregion volume differences for the ASD group were lost in comparison with the TD group; however, bilateral reduction in hippocampal volume among the DD group remained. Importantly, for the AD subgroup of more severely affected children, bilateral amygdalar enlargement was disproportionate to the increased cerebral volume. Our observations of amygdalar enlargement proportional to overall increased cerebral volume for the ASD group, and disproportional amygdalar en-

largement for the AD subgroup, are consistent with findings of one recent study of adults with autism, which found bilateral enlargement of the amygdalae, hypothesized to reflect incomplete neuronal pruning in early development.⁹ Pertinent to this observation, postmortem findings from adults with autism reveal increased cell packing density of the amygdala.²⁴ Although a convenient explanation for size increases, other investigators report normal or reduced size of these structures in samples of children and adults with autism.^{11-14,16} For example, a recent imaging study that evaluated seven men with autism, as part of a functional MRI activation study, found an approximate 15% bilateral reduction in amygdalar volume.¹¹ Another recent imaging study of adults with autism found no overall differences in amygdalar volume, although a subgroup having Asperger syndrome was observed to have relative enlargement.¹² From a small sample of 14 high-functioning adolescent and young adult men with autism, another group of investigators report decreased amygdalar volumes and a trend toward smaller hippocampi compared with normal control subjects.¹³ In that report, subregional volume differences between diag-

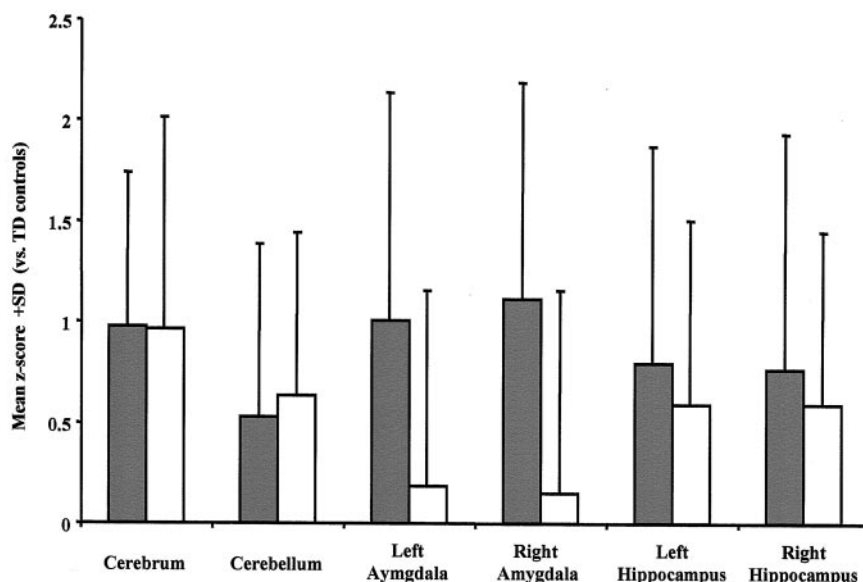


Figure 4. Volume relationships (\pm SD) normalized to the combined typically developing (TD) sample for autistic disorder (AD) subgroup (gray columns) and the subgroup with pervasive developmental disorder not otherwise specified (white columns).

nostic groups were accentuated, and reductions in hippocampal volume became significant when scaled by the ratio of combined cerebrum and cerebellum brain volume (target structure volume divided by total brain volume). Because the volumetric measurement methodology used in the current study was similar to that used in this earlier report in which decreased amygdalar size was found, and because reliability was established between investigators performing those measurements (E.H.A. and B.F.S.), further comment is warranted. In addition to the larger sample reported in our study, the age range and behavioral symptomatology of individuals evaluated was substantially different between studies; in the prior report, subjects were high-functioning (IQ of 80 or higher) with ages ranging between 11 and 37 years, whereas we specifically evaluated 3- to 4-year-old children across a broad functional range of strictly defined autism soon after the establishment of a clinical diagnosis. Because hippocampal and amygdalar volumes among the 3- to 4-year-olds evaluated as part of this study had not yet reached typical adolescent or adult size,^{13,35} individuals with autism may exhibit arrested development, or increased apoptosis, of these structures over time.²³ An additional consideration in reconciling these results is that autistic subgroups may have differences in amygdalar size. Differing findings in the literature may reflect the extent to which small adult samples are composed of mixed populations, reflecting more clinical heterogeneity than longitudinal progression per se.

The amygdala plays a crucial role in behavioral responses to emotional stimuli and in emotional learning.⁴⁴ In previous animal work, bilateral damage to the mesial temporal lobes of healthy adult monkeys was demonstrated to disrupt normal memory and emotional functions.⁴⁵ On closer analysis, amygdalar damage was most closely related to emotional dysfunction. This region, when lesioned selectively, impairs recognition of emotional faces⁴⁶ and has been implicated in an impaired ability to link visual perception of emotionally relevant stimuli among individuals with autism.⁴⁷ Our findings of a specific relationship for amygdalar abnormalities in children with more severe clinical expression of autism (AD vs PDD-NOS) are intriguing and suggest a relationship between amygdala and core symptoms of autism early in the clinical course. Whether these disparate amygdalar findings indicate a different clinical course of the disorder will be investigated at follow-up and with detailed integration of neuropsychological and structural findings in future reports.

We evaluated neuroimaging findings early in the clinical course of autism. The diagnostic agreement between the formal clinician-determined algorithm used for diagnosis (ADOS-G), parental report (ADI-R), and clinical judgment based on observation and history is considered to be good by 3 years of age.⁴⁸ However, individual children may exhibit changing symptom expression over time, which would affect

their fulfilling diagnostic criteria, particularly those criteria distinguishing AD from PDD-NOS. Because these children are being followed longitudinally and will be reimaged at 6 to 7 years of age, it will be possible to determine whether some of the children have fewer or more symptoms at the end of the pre-school period and, moreover, whether such changes are related to the progression of brain morphometric findings.

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