

# Amygdala Enlargement in Toddlers with Autism Related to Severity of Social and Communication Impairments

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**Background:** Autism is a heterogeneous neurodevelopmental disorder of unknown etiology. The amygdala has long been a site of intense interest in the search for neuropathology in autism, given its role in emotional and social behavior. An interesting hypothesis has emerged that the amygdala undergoes an abnormal developmental trajectory with a period of early overgrowth in autism; however this finding has not been well established at young ages nor analyzed with boys and girls independently.

**Methods:** We measured amygdala volumes on magnetic resonance imaging scans from 89 toddlers at 1–5 years of age (mean = 3 years). Each child returned at ~5 years of age for final clinical evaluation.

**Results:** Toddlers who later received a confirmed autism diagnosis (32 boys, 9 girls) had a larger right ( $p < .01$ ) and left ( $p < .05$ ) amygdala compared with typically developing toddlers (28 boys, 11 girls) with and without covarying for total cerebral volume. Amygdala size in toddlers with autism spectrum disorder correlated with the severity of their social and communication impairments as measured on the Autism Diagnostic Interview and Vineland scale. Strikingly, girls differed more robustly from typical in amygdala volume, whereas boys accounted for the significant relationship of amygdala size with severity of clinical impairment.

**Conclusions:** This study provides evidence that the amygdala is enlarged in young children with autism; the overgrowth must begin before 3 years of age and is associated with the severity of clinical impairments. However, neuroanatomic phenotypic profiles differ between males and females, which critically affects future studies on the genetics and etiology of autism.

**Key Words:** Brain growth, development, MRI, neuroanatomy, temporal, volume

The amygdala has long been a site of intense interest in the search for neuropathologic markers for autism, given its well-established role in the production and recognition of emotions and modulatory role in social behavior (1). Initial signs of autism in toddlers include unusual affective behavior, reduced social interest, and poor eye contact (2,3), which are all suggestive of aberrant amygdala function. In addition, the core behavioral features of the autism diagnosis at 4 years of age are similarly suggestive of amygdala dysfunction, including impairments in reciprocal social interaction and abnormal development of nonverbal communication (4). In addition, common comorbid neurological disorders (5,6) such as epilepsy and anxiety may be attributed to an abnormally functioning amygdala (7,8).

The heterogeneity and unknown etiologies of autism, variability of analyses employed, and different age groups studied have limited the consistency of results across structural magnetic resonance imaging (MRI) studies of amygdala volume (9). In particular, consideration of subject age has emerged as a critical factor (10). Only three studies have been published on the size of the amygdala in children with autism aged less than 10 years, finding the amygdala to be enlarged by approximately 15% in children with confirmed autism relative to age-matched control subjects (10–12). However, studies of older adolescents, adults,

or a wide age range of subjects have found either no difference in (13) or even smaller (14–16) amygdala volumes in individuals with autism. In addition, amygdala volume increases throughout adolescence in typically developing male children (17,18) but not in male children with autism (10). Thus, an intriguing hypothesis has emerged that the amygdala is initially larger than normal in children with autism but then does not undergo the same age-related increase in volume that takes place in typically developing children. This hypothesis parallels the general theory of early brain overgrowth in young children with autism (19,20), although the aberrant growth trajectory of the amygdala may extend to a later age of development relative to cortical regions (10). Several questions remain: At what age in development does the amygdala become enlarged in children with autism? Is the enlargement related to the degree of social and emotional impairments? Do males and females with autism differ in their neuroanatomical phenotype?

The objectives of this study were to measure the volume of the amygdala in toddlers at risk for autism compared with age-matched typically developing children at the age of first clinical detection, to evaluate the potential relationship between amygdala size and the severity of behavioral impairments at final clinical outcome, and to characterize the neuropathologic and behavioral profiles of males and females independently.

## Methods and Materials

### Diagnostic Assessment

Eighty-nine toddlers ( $n = 66$  males, 23 females) between the ages of 18 and 60 months were included in amygdala volumetric analyses as part of a longitudinal MRI study of early brain development in autism (Table 1). A parent or guardian gave informed consent for participation in this research program as approved by the Institutional Review Board of Rady Children's Hospital San Diego and the University of California, San Diego.

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**Table 1.** Subject and Diagnostic Data at Final Clinical Visit

	Autism ( <i>n</i> = 41)		PDD-NOS ( <i>n</i> = 9)		Typical ( <i>n</i> = 39)	
	Male	Female	Male	Female	Male	Female
Number of Subjects	32	9	6	3	28	11
Mean Age at MRI (Months)	36 ± 7.2	36 ± 4.7	36 ± 9.1	56 ± 6.1	34 ± 7.1	37 ± 6.4
Age Range at MRI (Months)	22–54	26–44	25–50	49–61	20–51	23–44
Mean Age at Final Clinical Visit (Months)	49 ± 1.1	49 ± 9.7	59 ± 9.8	50 ± 7.9	46 ± 1.3	46 ± 1.2
Full-Scale IQ	58 ± 20	57 ± 23	93 ± 32	63 ± 19	111 ± 17	115 ± 15
Verbal IQ	50 ± 20	50 ± 29	82 ± 27	56 ± 13	113 ± 19	115 ± 15
Performance IQ	65 ± 19	62 ± 21	104 ± 35	72 ± 31	109 ± 20	113 ± 16
ADI-R Social	19 ± 6	18 ± 6	14 ± 5	15 ± 2	NA	NA
ADI-R NV Comm.	10 ± 3	9 ± 3	6 ± 3	7 ± 5	NA	NA
ADI-R RnR	6 ± 2	5 ± 2	5 ± 3	4 ± 2	NA	NA
ADOS Social	10 ± 3	11 ± 2	5 ± 2	5 ± 2	NA	NA
ADOS Communication	6 ± 2	7 ± 2	4 ± 2	5 ± 1	NA	NA
ADOS SBRI	3 ± 1	3 ± 2	1 ± 1	4 ± 2	NA	NA
Vineland Social	66 ± 9	64 ± 7	78 ± 9	71 ± 8	97 ± 8	102 ± 15
Vineland Communication	66 ± 11	62 ± 16	84 ± 16	69 ± 11	108 ± 11	105 ± 9
Vineland ABC	63 ± 8	64 ± 11	74 ± 14	67 ± 15	102 ± 10	105 ± 12

ABC, Adaptive Behavior Composite; ADI-R, Autism Diagnostic Interview—Revised; ADOS, Autism Diagnostic Observation Schedule; MRI, magnetic resonance imaging; NV Comm., nonverbal communication; PDD-NOS, pervasive developmental disorder—not otherwise specified; RnR, restrictive and repetitive; SBRI, stereotyped behaviors and restricted interests.

Provisionally autistic 12- to 36-month-old children were initially recruited by clinician referral, through presentations at local autism support group meetings, and by letters distributed at agencies (directed to parents of children with autism who may have younger siblings). Typically developing young children were recruited through notices given to local preschools, magazine advertisements, and referral from parents already participating.

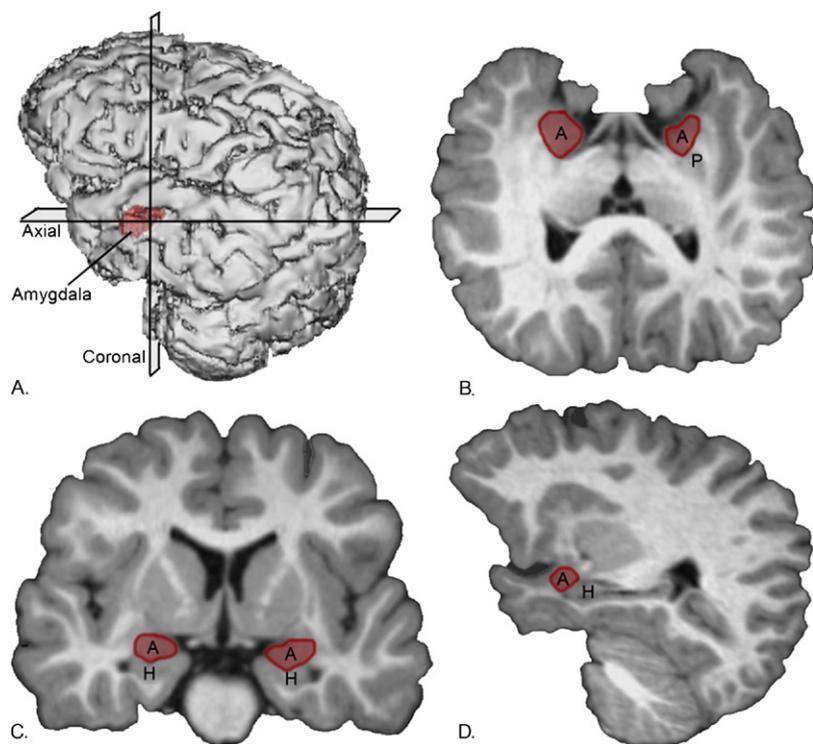
At entry into the study between 12 and 36 months of age, children received a diagnostic evaluation by a research psychologist (CCB) with extensive clinical experience with autism. During the initial visit, the Mullen Scales of Early Learning (21) was administered as a standardized measure of early cognition. Toddlers (*n* = 63 males, 18 females) who displayed symptoms of autism were assigned to a “provisional autism spectrum disorder” (p-ASD) group and administered the Autism Diagnostic Observation Schedule (ADOS) (22,23); their parents completed the Autism Diagnostic Interview—Revised (ADI-R) (24). The ADOS is a semistructured standardized assessment of communication, social interaction, and play in young children to evaluate presence of autism spectrum disorder. It provides a diagnostic classification of *autism*, *autism spectrum*, or *nonspectrum* based on the child’s presentation during the assessment. The ADI-R is a standardized comprehensive parent interview designed to assess, confirm, or negate an autism spectrum disorder through queries closely associated with the DSM-IV (4). Toddlers (*n* = 28 males, 11 females) who presented age-equivalent performance on the Mullen Scales and showed no characteristics of autism were judged as typically developing and placed in the “control” group. Toddlers who showed delays on the standardized cognitive assessment but did not meet criteria for autism spectrum disorder were not included in the current analyses. Each child returned at approximately 36 months of age for an MRI scan and again at approximately 48 months of age for the final clinical evaluation. At the final visit, the ADOS, ADI-R, either the Mullen or the Wechsler Preschool and Primary Scale of Intelligence—III (25) (depending on child’s age), and the Vineland Adaptive Behavioral Scales Survey Interview (26) were administered. The Vineland is a standardized parent or caregiver interview used to assess a child’s functional skills in four developmental domains; this

was administered to both the control and p-ASD groups. Only the exit ADI-R (standardized for age 4) and Vineland scores were used for statistical and correlation analyses in this study.

At 48 months or older, children in the p-ASD group were given a final diagnosis (by authors CCB and CL) of autistic disorder (*n* = 32 males, 9 females), pervasive developmental disorder—not otherwise specified (PDD-NOS; *n* = 6 males, 3 females), or nonspectrum based on the exit ADI-R and final ADOS (Table 1). Fifteen males and two females with a provisional autism diagnosis had at least one successful MRI scan but dropped from the study before final diagnosis could be determined. Ten males and four females in the p-ASD group who completed the final diagnostic assessment were considered nonspectrum at final diagnosis. Of these, four males and two females were given a developmental delay diagnosis, and the others were mixed nonspectrum (e.g., attention-deficit/hyperactivity disorder, receptive-expressive language disorder). Neither participants who dropped out before final diagnosis nor those who did not meet criteria for autism spectrum disorder at final evaluation were included in Table 1 or in amygdala volumetric analyses.

### Neuroimaging

All MRI scans were collected at the UCSD Medical Center, Hillcrest, on a 1.5-Tesla Siemens Symphony system (Siemens, Malvern, Pennsylvania). Thirty-three p-ASD (25 autism, 8 PDD-NOS) study participants received sedation (propofol) to undergo MRI. None of the typically developing control subjects underwent anesthesia. All nonsedation scans were performed at night during natural sleep. The protocols for scanning each participant included a three-dimensional T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE; 128 coronal slices, matrix 224 × 256 mm, .859375 mm × .859375 mm in-plane × 1.5 mm slice thickness, echo time = 3.67 msec, repetition time = 2730 msec). Although this study was part of a larger longitudinal study of brain development in autism and many subjects in this cohort received scans at multiple time points, only the scan collected closest to 36 months of age (Table 1) was used for this analysis of amygdala volume.



**Figure 1.** Three-dimensional reconstruction of images (A) in which lines indicate the position through the cerebrum of the transverse (or axial) plane (B), coronal plane (C), and sagittal plane (D). A, amygdala; H, hippocampus; P, putamen.

Scans were transferred to the laboratory where each MPRAGE sequence was first used to measure total cerebral volume (TCV), which comprised total cortical gray and white matter volumes and excluded subcortical structures. The boundaries of the cerebrum were manually defined as previously described in detail (27,28) using the software program AREA (developed by Matthew Belmonte [29]).

To measure amygdala volume, each raw MPRAGE sequence was imported into the program Analyze 8.1 (30) and processed in an identical manner to procedures described in Schumann *et al.* (10). Briefly, images were converted to .5-mm cubic voxel dimensions and reoriented along the horizontal axis from rostral to caudal pole of the hippocampus to aid in distinguishing the amygdala from the hippocampus (Figure 1A). Two blinded raters manually traced the amygdala after establishing reliability on the MRI scans of 20 subjects with an interrater reliability correlation of  $>.94$  and an intrarater reliability correlation of  $>.95$ . The initial tracing process involved defining the borders in coronal sections starting with the most caudal level in which the amygdala was visible to the approximate most rostral section in which the amygdala was present (Figure 1B). Outlines were then verified and edited in the horizontal (axial) and sagittal views that were simultaneously available to the rater while tracing (Figure 1C and 1D).

### Statistics

Statistical analyses were performed with Statistical Program for the Social Sciences Edition 16.0 software (SPSS, Chicago, Illinois). Differences between study diagnostic groups for age of the participant at the time of the MRI scan and clinical scores were tested using analysis of variance (ANOVA). An analysis of covariance (ANCOVA) was performed to compare amygdala volume between the autism and control groups after adjusting for the covariates of sex and TCV. The ANCOVA was then repeated for males and females separately. To detect a potential relationship of amygdala volume with clinical diagnostic scores,

partial correlation analyses were carried out independently for two participant groups: 1) autism spectrum disorder (ASD), which included subjects with a confirmed diagnosis of autism and those with PDD-NOS, and 2) typically developing age-matched control subjects. The age of the participant at the time of scan and at the final clinical visit were considered covariates. Males and females were also analyzed independently.

### Results

Clinical diagnostic and behavioral measures collected at final clinical visit are listed in Table 1. There was no difference in the age of the groups at the time of MRI acquisition. There was, as expected, a significant group effect for all IQ and Vineland measures ( $p < .001$ ).

Mean volumetric data for all participants are given in Table 2. Amygdala volumes in all groups significantly correlated with age at time of scan ( $r = .49$ ,  $p < .05$ ); however, there was no age  $\times$  group effect for autism and control subjects, and therefore age was not included as a covariate in further volumetric comparisons. ANCOVA with sex as a covariate revealed increased right [ $F(1,77) = 12.39$ ,  $p = .001$ ] and left [ $F(1,77) = 6.83$ ,  $p = .01$ ] amygdala volume in the autism group relative to control subjects. There was no significant difference in TCV between the autism and control groups and no TCV  $\times$  group interaction. When sex and TCV were included as covariates, right [ $F(1,76) = 10.19$ ,  $p = .002$ ] and left [ $F(1,76) = 4.95$ ,  $p = .03$ ] amygdala volumes were significantly enlarged in the autism group relative to control subjects. Amygdala volumes in subjects with PDD-NOS were not analyzed independently because there were too few subjects to justify a separate group.

When males and females were analyzed separately (Figure 2), males with autism had a significantly larger right [ $F(1,58) = 6.46$ ,  $p = .014$ ] and a trend toward a larger left [ $F(1,58) = 3.12$ ,  $p = .08$ ] amygdala volume compared with age-matched typically developing males. There was a medium effect size of .63 and .44 for

**Table 2.** Volumetric Measures (in Cubic Centimeters)  $\pm$  Standard Deviations for All Participants by Diagnostic Group and Sex

	Autism (n = 41)		PDD-NOS (n = 9)		Typical (n = 39)	
	Male	Female	Male	Female	Male	Female
Right Amygdala	1.84 $\pm$ .19 <sup>a</sup>	1.47 $\pm$ .09 <sup>b</sup>	1.62 $\pm$ .06	1.45 $\pm$ .14	1.73 $\pm$ .16	1.31 $\pm$ .07
Left Amygdala	1.77 $\pm$ .21	1.40 $\pm$ .14 <sup>b</sup>	1.62 $\pm$ .12	1.39 $\pm$ .16	1.69 $\pm$ .15	1.26 $\pm$ .07
Total Cerebral	1090 $\pm$ 81	1046 $\pm$ 67	1079 $\pm$ 88	1015 $\pm$ 104	1071 $\pm$ 88	1003 $\pm$ 78

Autism subjects significantly differed from sex-matched control subjects. Subjects with pervasive developmental disorder—not otherwise specified (PDD-NOS) were not included in volumetric comparisons because of limited sample size.

<sup>a</sup> $p < .05$ .

<sup>b</sup> $p < .01$ .

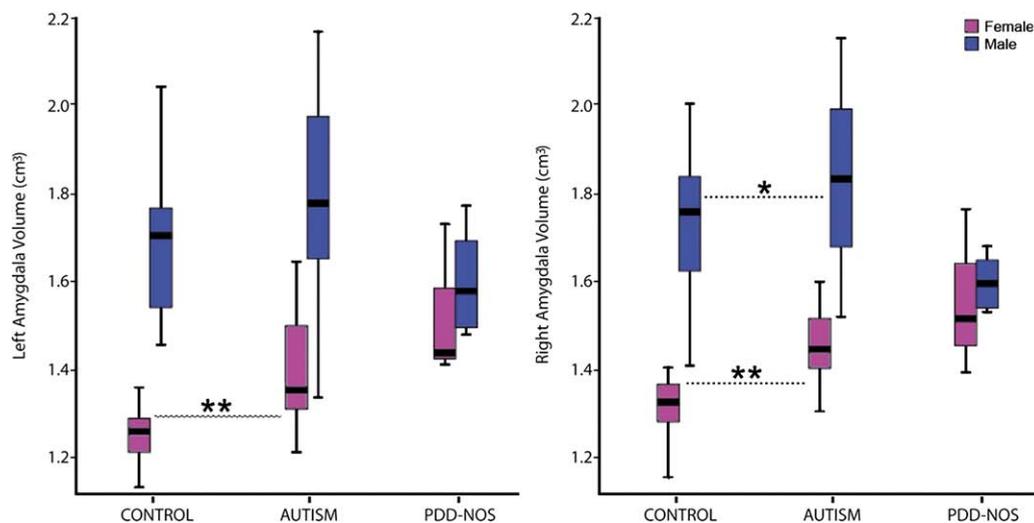
left and right amygdala volume, respectively, indicating sufficient power to detect a difference between autism and control male groups. When TCV was included as a covariate, right [ $F(1,57) = 5.65, p = .021$ ] amygdala volume remained significantly enlarged in males with autism compared with control subjects.

Females with autism had a significantly larger right [ $F(1,18) = 16.71, p = .001$ ] and left [ $F(1,18) = 8.98, p = .008$ ] amygdala volume compared with age-matched typically developing females. When TCV was included as a covariate, right [ $F(1,17) = 13.23, p = .002$ ] and left [ $F(1,17) = 6.74, p = .019$ ] amygdala volume remained significantly enlarged in females with autism compared with control subjects.

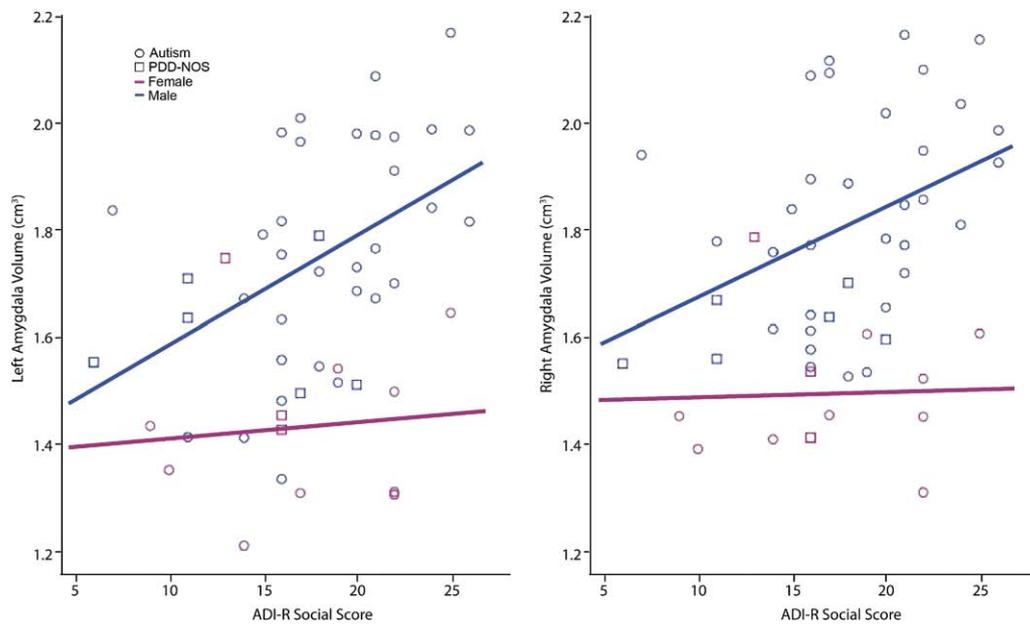
For analyses of the relationship of amygdala volume to clinical scores, the autism and PDD-NOS groups were combined to form an ASD group. There were significant age at scan  $\times$  group and age at final clinical evaluation  $\times$  group interactions for ASD relative to control; therefore, age at scan and at the time of clinical evaluation were included as covariates. Partial correlation analyses, with sex and age as covariates, revealed a significant correlation of the ADI-R Social score of ASD participants with their right ( $r = .44, p = .002$ ) and left ( $r = .48, p = .001$ ) amygdala volumes. When sex was analyzed separately, right ( $r = .52, p = .001$ ) and left ( $r = .57, p < .001$ ) amygdala volumes were correlated with the ADI-R Social scores of ASD males (Figure 3). The ADI-R Nonverbal Communication score also significantly correlated with the left amygdala volume ( $r = .48, p = .004$ ) in ASD males. ASD females did not show detectable correlations

between amygdala volume and any clinical measures. ASD amygdala volumes, either combined or for males and females independently, did not correlate with the ADI-R Restricted, Repetitive, and Stereotyped Patterns of Behavior score. Total cerebral volume did not significantly correlate with any of the measures in any groups but showed a trend toward correlating with the ADI Social score ( $p = .08$ ) and Repetitive Behavior score ( $p = .07$ ) for ASD subjects covaried for age at scan, age at diagnosis, and sex.

When all subjects were analyzed together and covaried for sex, age at scan, and age at final clinical visit on the Vineland, there was a significant negative correlation of communication score with right ( $r = -.31, p < .01$ ) and left ( $r = -.30, p < .01$ ) amygdala volume (Figure 4) and the social score with right ( $r = -.31, p < .01$ ) and left ( $r = -.31, p < .01$ ) amygdala volume. When control subjects were analyzed independently, they did not show significant correlations between amygdala volume and any clinical measures. When ASD subjects were analyzed independently, they showed a significant negative correlation in Vineland Communication and Social scores and right ( $r = -.31, p < .05$ ) and left ( $r = -.30, p < .05$ ) amygdala volumes. In ASD males but not ASD females, there was a significant negative correlation in Vineland Communication score and right ( $r = -.38, p < .05$ ) and left ( $r = .34, p < .05$ ) amygdala volume. The Vineland Social score also negatively correlated with right ( $r = -.35, p < .05$ ) and left ( $r = -.34, p = .05$ ) amygdala volume in ASD males but not ASD females.



**Figure 2.** Amygdala volume (in cubic centimeters) for all participants by diagnostic group ( $*p < .05$ ;  $**p < .01$  significantly different from sex-matched control subjects). Length of box is the interquartile range computed from Tukey's hinges. The line inside the box is median, and whiskers represent the entire value range. PDD-NOS, pervasive developmental disorder—not otherwise specified.



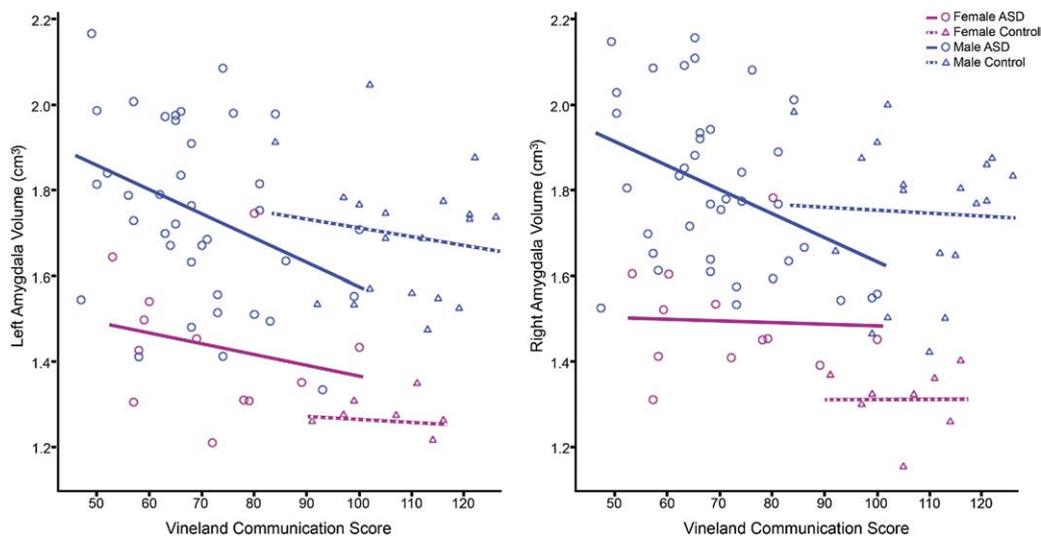
**Figure 3.** Linear regression scatter plots showing a positive correlation for right ( $r = .52, p = .001$ ) and left ( $r = .57, p < .001$ ) amygdala volume (in cubic centimeters) and Autism Diagnostic Interview—Revised (ADI-R) social score in males with autism spectrum disorder. PDD-NOS, pervasive developmental disorder—not otherwise specified.

**Discussion**

This study provides evidence that the amygdala is enlarged in young children with autism and that the overgrowth must begin before 3 years of age at approximately the time symptoms become clinically evident. This finding is consistent with early overgrowth found in cortical regions (19,20) but differs in that the amygdala may remain enlarged later into childhood than the rest of the brain (10). Additionally, the enlargement in right amygdala volume in males and females and left amygdala volume in females is disproportionate to total cerebral volume at 3 years. In male toddlers who eventually receive a diagnosis of autism spectrum disorder, the degree of amygdala enlargement at that early age is associated with the severity of their social and communication impairments at final clinical evaluation at ~5

years of age. This is also similar to findings from other brain regions, in which enlargement at 3 to 5 years of age is associated with the severity of symptoms at 5 to 8 years of age (31).

Our study is the first to report the striking finding that amygdala enlargement in females with autism is more severe, compared with age- and sex-matched typical-developing counterparts, than in males with autism. Indeed, a significant difference was present despite a group size of only nine females with autism. Interestingly, unlike ASD males, the enlargement in ASD females was not related to the severity of social and communication impairments, although this may simply reflect the modest sample size. Previous studies (32) have also found more robust differences in cortical volumes in females than males. Although speculative at present, this suggests that autistic males may be a



**Figure 4.** Linear regression scatter plots showing a positive correlation for right ( $r = -.38, p < .05$ ) and left ( $r = .34, p < .05$ ) amygdala volumes (in cubic centimeters) and Vineland communication score in males with autism spectrum disorder (ASD).

more heterogeneous group in amygdala volume, which varies with the degree of clinical impairment, compared with autistic females, who may have a more homogeneous neuropathologic profile of an enlarged amygdala regardless of the degree of their behavioral impairment. This finding awaits confirmation from a larger study on females with autism but also raises further questions regarding sex differences: Does amygdala enlargement in females represent a completely different neuropathologic process, or is the same process altered by other components of the social brain in females with autism? Might pathology in different brain regions be associated with the social behavior impairments in females with ASD?

Our findings of an enlarged amygdala are similar to that of Sparks and colleagues (12) and the recently published study by Mosconi and colleagues (11), which to date are the only other published studies on amygdala volume in young children aged under 5 years with a confirmed diagnosis of autism. In the Sparks study, the 4-year-old participants with autism had enlarged amygdala volumes bilaterally compared with age-matched typically developing children. In the Mosconi study, bilateral amygdala enlargement was observed in children with autism at both 2 and 4 years of age compared with a control group that consisted of typical and developmentally delayed children. However, neither study analyzed males and females with a confirmed diagnosis of autism independently; Sparks *et al.* did analyze females with ASD and did not find a difference in amygdala volume compared with control subjects, but this small group of  $n = 7$  females included only three with autism. These differences limit the viability of a direct comparison between studies. Munson *et al.* (33) examined the relationship between amygdala volume and the severity of clinical outcome in the Sparks *et al.* (12) cohort. Similar to our findings, they reported that a larger right amygdala volume at 3 to 4 years of age was associated with more severe social and communication impairments on the ADI and Vineland and poorer outcome at 6 years. However, they did not evaluate males and females independently.

One inherent limitation of our study is that the autism group had significantly lower cognitive abilities, as demonstrated with lower IQ scores, than the control group. It is possible that pathology in the amygdala is not specific to autism but common to mental retardation. This is doubtful, however, because studies that include subjects with mental retardation without autism (12,34,35) indicate that the amygdala is not enlarged, as we find in our autism sample, but instead are similar in size to typically developing control subjects. Therefore, the findings of our study are unlikely to be driven solely by low cognitive ability in children with autism.

Taken together with similar previously published studies, our study confirms that the amygdala is enlarged in young children with autism (10,12,36) and that the period of enlargement appears to be limited to early development given the continued growth of the amygdala, which increases in size by 40% from 5 years to 18 years, in typically developing males (17,18). Therefore, by adolescence, amygdala size in the typically developing child has caught up with, and likely surpassed, amygdala size in the child with autism (10). Studies of older populations have found that amygdala volume is smaller in autism relative to age-matched typical control subjects (14–16). In one study by Nacewicz and colleagues (15), a smaller amygdala volume was associated with gaze avoidance and more severe behavioral impairments as measured with the ADI-R. This relationship is the opposite pattern of that observed in younger children in the current study as well as Munson *et al.* (33). It remains unknown

whether autism subjects that demonstrate early overgrowth also demonstrate reduced amygdala size in adulthood, as preliminarily suggested by these findings; this awaits confirmation from a longitudinal study on brain growth throughout development in people with autism.

How might pathology in the amygdala relate to specific behavioral abnormalities? We and others (15,33) have found that the volume of the amygdala in individuals with ASD is associated with the severity of their social and nonverbal impairments as measured on the ADI and Vineland. The correlation analyses carried out in this study are considered exploratory, and few specific inferences may be drawn linking amygdala pathology to specific behaviors scored on these tests because each score comprises several behavioral measures. Additionally, there is considerable overlap between the behaviors that comprise the social and nonverbal communication scores on both algorithms. Both scores include amygdala-associated behaviors such as observing social smiling, gaze direction, facial expressions, imaginative and imitative social play, and seeking to share enjoyment with others. Other lines of research, such as functional imaging in older children and adults with autism, provide some evidence of an abnormal pattern of amygdala activation in response to social stimuli.

In Pierce and colleagues (37) and Schultz (38), it was suggested that abnormalities in the amygdala in autism, as well as diminished attention to faces early on (39), may be the first in a cascade of problems that lead to later emotional and social impairments. An emerging hypothesis is that the amygdala may play a role in mediating or directing visual attention to the eye region of the face to detect emotion or danger (1). However, autism subjects show abnormal visual scan paths during eye-tracking studies when viewing faces, typically spending little time on core social features such as the eyes (40,41). As mentioned earlier, Nacewicz *et al.* (15) found that individuals with autism (8–25 years of age) who had a smaller amygdala also spent the least amount of time fixating on the eye region of the face. It is unclear whether this finding in people with autism reflects a lack of motivation or anxiety (or both) related to looking at the eyes for social and emotional cues. Pierce and colleagues (37) found that when autistic subjects viewed familiar faces, they were able to activate the amygdala appropriately in response to both familiar and unfamiliar faces, suggesting that the familiar faces may have enhanced motivation or attention to all the stimuli. Dalton and colleagues (42) used functional imaging and eye-tracking technology simultaneously while showing subjects familiar and unfamiliar faces; the amount of time persons with autism spent looking at the eye region of the face was strongly positively correlated with amygdala activation, but not in typically developing control subjects. This suggests a heightened emotional, or even fearful, response when autistic individuals look at another person's eyes, regardless of whether they are familiar or a stranger. Spezio *et al.* (43) confirmed that participants with autism show less fixation on the eyes and mouth, but also a greater tendency to saccade away from the eyes when information was present in those regions. Recently, Kleinhans and colleagues (44) found evidence of reduced amygdala habituation to faces in their autism spectrum group; the degree of reduction was related to the severity of social impairments, which lends further evidence to the theory that the amygdala is hyperaroused in people with autism in response to socially relevant stimuli.

The amygdala, with its dense reciprocal connections with the visual stream (45), modulates many levels of visual processing

that in turn may influence the development of early preference for faces seen in typical newborns (46). However, the amygdala is just one of several structures that work in parallel to produce normal social cognition, one of the core impairments in autism. The amygdala, when presented with a socially demanding situation, evaluates and allocates resources to those stimuli that pose a potential threat. Structures such as the fusiform gyrus, frontal cortex, cingulate cortex, somatosensory cortex, and the superior temporal gyrus also have specialized roles in the broader system of social behavior (47), and the detection of potential neuropathology in these regions, as well as how each might be influenced by the amygdala, is an important direction for future study.

In conclusion, we found an enlarged amygdala volume in toddlers with autism; the degree of amygdala enlargement at that age was associated with the severity of social and communication impairments at 5 years of age. The amygdala was disproportionately enlarged relative to total brain volume in the toddlers with autism. Males and females differed in their neuropathologic and behavioral profiles; females more robustly differed from typical in amygdala volume and males showed a significant relationship of amygdala size with the severity of their social and communication impairments. However, many factors contribute to the volume of a structure, including the number and size of neurons and glia, dendritic arborization, myelination, vasculature, and so forth, and therefore more research is required to explain the unusual amygdala growth trajectory that has been further supported by our findings. It is clear, however, that the age and sex of the participants should be considered essential factors in evaluating and interpreting findings of all future volumetric studies of autism. These findings will have a major impact on defining the neural phenotypes of males and females with autism, which is critical for the next phase of etiologic and genetic phenotyping research.

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- Adolphs R (2008): Fear, faces, and the human amygdala. *Curr Opin Neurobiol* 18:166–172.
- Dawson G, Meltzoff AN, Osterling J, Rinaldi J (1998): Neuropsychological correlates of early symptoms of autism. *Child Dev* 69:1276–1285.
- Werner E, Dawson G, Osterling J, Dinno N (2000): Brief report: Recognition of autism spectrum disorder before one year of age: A retrospective study based on home videotapes. *J Autism Dev Disord* 30:157–162.
- American Psychiatric Association (1994): *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* Washington, DC: American Psychiatric Association.
- Amaral DG, Schumann CM, Nordahl CW (2008): Neuroanatomy of autism. *Trends Neurosci* 31:137–145.
- DiCicco-Bloom E, Lord C, Zwaigenbaum L, Courchesne E, Dager SR, Schmitz C, *et al.* (2006): The developmental neurobiology of autism spectrum disorder. *J Neurosci* 26:6897–6906.
- Bishop SJ (2007): Neurocognitive mechanisms of anxiety: An integrative account. *Trends Cogn Sci* 11:307–316.
- Pitkanen A, Tuunanen J, Kalviainen R, Partanen K, Salmenpera T (1998): Amygdala damage in experimental and human temporal lobe epilepsy. *Epilepsy Res* 32:233–253.
- Schumann CM, Amaral DG (2009): The human amygdala and autism. In: Whalen P, Phelps E, editors. *The Human Amygdala*. New York: Guilford Press.
- Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, *et al.* (2004): The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *J Neurosci* 24:6392–6401.
- Mosconi MW, Cody-Hazlett H, Poe MD, Gerig G, Gimpel-Smith R, Piven J (2009): Longitudinal study of amygdala volume and joint attention in 2- to 4-year-old children with autism. *Arch Gen Psychiatry* 66:509–516.
- Sparks BF, Friedman SD, Shaw DW, Aylward E, Echelard D, Artru AA, *et al.* (2002): Brain structural abnormalities in young children with autism spectrum disorder. *Neurology* 59:184–192.
- Haznedar MM, Buchsbaum MS, Wei TC, Hof PR, Cartwright C, Bienstock CA, *et al.* (2000): Limbic circuitry in patients with autism spectrum disorders studied with positron emission tomography and magnetic resonance imaging. *Am J Psychiatry* 157:1994–2001.
- Aylward EH, Minshew NJ, Goldstein G, Honeycutt NA, Augustine AM, Yates KO, *et al.* (1999): MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology* 53:2145–2150.
- Nacewicz BM, Dalton KM, Johnstone T, Long MT, McAuliff EM, Oakes TR, *et al.* (2006): Amygdala volume and nonverbal social impairment in adolescent and adult males with autism. *Arch Gen Psychiatry* 63:1417–1428.
- Pierce K, Müller R-A, Ambrose J, Allen G, Courchesne E (2001): Face processing occurs outside the fusiform “face area” in autism: Evidence from fMRI. *Brain* 124:2059–2073.
- Giedd JN (1997): Normal development. *Child Adolesc Psychiatr Clin North Am* 6:265–282.
- Giedd JN, Snell JW, Lange N, *et al.* (1996): Quantitative magnetic resonance imaging of human brain development: Ages 4–18. *Cerebral Cortex* 6:551–560.
- Courchesne E, Karns CM, Davis HR, Ziccardi R, Carper RA, Tigue ZD, *et al.* (2001): Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology* 57:245–254.
- Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, *et al.* (2007): Mapping early brain development in autism. *Neuron* 56:399–413.
- Mullen EM (1995): *Mullen Scales of Early Learning*. Circle Pines, MN: American Guidance Service.
- DiLavore PC, Lord C, Rutter M (1995): The pre-linguistic autism diagnostic observation schedule. *J Autism Dev Disorders* 25:355–379.
- Lord C, Risi S, Lambrecht L, Cook E Jr, Leventhal B, DiLavore P, *et al.* (2000): The Autism Diagnostic Observation Schedule—Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev 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. *J Autism Dev Disorders* 24:659–685.
- Wechsler D (2002): *Wechsler Preschool and Primary Scale of Intelligence—III*. San Antonio, TX: Psychological Corporation.
- Sparrow S, Balla D, Cicchetti D (1984): *Vineland Scales of Adaptive Behavior: Survey Form Manual*. Circle Pines, MN: American Guidance Service.
- Carper RA, Moses P, Tigue ZD, Courchesne E (2002): Cerebral lobes in autism: Early hyperplasia and abnormal age effects. *Neuroimage* 16:1038–1051.
- Courchesne E, Karns C, Davis HR, Ziccardi R, Carper R, Tigue Z, *et al.* (2001): Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology* 57:245–254.
- Egaas B, Courchesne E, Saitoh O (1995): Reduced size of corpus callosum in autism. *Arch Neurol* 52:794–801.
- Robb RA, Hanson DP, Karwoski RA, Larson AG, Workman EL, Stacy MC (1989): Analyze: A comprehensive, operator-interactive software pack-

- age for multidimensional medical image display and analysis. *Comput Med Imaging Graph* 13:433–454.
31. Akshoomoff N, Lord C, Lincoln A, Courchesne RY, Carper R, Townsend J, *et al.* (2004): Outcome classification of preschool children with autism spectrum disorders using MRI brain measures. *J Am Acad Child Adolesc Psychiatry* 43:349–357.
  32. Bloss CS, Courchesne E (2007): MRI neuroanatomy in young girls with autism: A preliminary study. *J Am Acad Child Adolesc Psychiatry* 46:515–523.
  33. Munson J, Dawson G, Abbott R, Faja S, Webb SJ, Friedman SD, *et al.* (2006): Amygdalar volume and behavioral development in autism. *Arch Gen Psychiatry* 63:686–693.
  34. Pinter JD, Brown WE, Eliez S, Schmitt JE, Capone GT, Reiss AL (2001): Amygdala and hippocampal volumes in children with Down's syndrome: A high-resolution MRI study. *Neurology* 56:972–974.
  35. Zeegers M, Pol HH, Durston S, Nederveen H, Schnack H, Daalen EV, *et al.* (2008): No differences in MR-based volumetry between 2- and 7-year-old children with autism spectrum disorder and developmental delay [published online ahead of print Dec 8]. *Brain Dev.*
  36. Mosconi MW, Hazlett HC, Poe MD, Gerig G, Smith RG, Piven J (2009): A longitudinal study of amygdala volume and joint attention in 2–4 year old children with autism. *Arch Gen Psychiatry* 66:509–516.
  37. Pierce K, Haist F, Sedaghat F, Courchesne E (2004): The brain response to personally familiar faces in autism: Findings of fusiform activity and beyond. *Brain* 127:2703–2716.
  38. Schultz RT (2005): Developmental deficits in social perception in autism: The role of the amygdala and fusiform face area. *Int J Dev Neurosci* 23:125–141.
  39. Osterling J, Dawson G (1994): Early recognition of children with autism: A study of first birthday home videotapes. *J Autism Dev Disord* 24:247–257.
  40. Klin A, Jones W, Schultz R, Volkmar F, Cohen D (2002): Visual fixation patterns during viewing of naturalistic social situations as predictors of social competence in individuals with autism. *Arch Gen Psychiatry* 59:809–816.
  41. Pelphrey KA, Sasson NJ, Reznick JS, Paul G, Goldman BD, Piven J (2002): Visual scanning of faces in autism. *J Autism Dev Disord* 32:249–261.
  42. Dalton KM, Nacewicz BM, Johnstone T, Schaefer HS, Gernsbacher MA, Goldsmith HH, *et al.* (2005): Gaze fixation and the neural circuitry of face processing in autism. *Nat Neurosci* 8:519–526.
  43. Spezio ML, Adolphs R, Hurley RS, Piven J (2007): Abnormal use of facial information in high-functioning autism. *J Autism Dev Disord* 37:929–939.
  44. Kleinmans NM, Johnson LC, Richards T, Mahurin R, Greenson J, Dawson G, *et al.* (2009): Reduced neural habituation in the amygdala and social impairments in autism spectrum disorders. *Am J Psychiatry* 166:467–475.
  45. Amaral DG, Price JL (1984): Amygdalocortical projections in the monkey (*Macaca fascicularis*). *J Comp Neurol* 230:465–496.
  46. Johnson MH, Dziurawiec S, Ellis H, Morton J (1991): Newborns' preferential tracking of face-like stimuli and its subsequent decline. *Cognition* 40:1–19.
  47. Adolphs R (2001): The neurobiology of social cognition. *Curr Opin Neurobiol* 11:231–239.