

# Longitudinal Study of Amygdala Volume and Joint Attention in 2- to 4-Year-Old Children With Autism

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**Context:** Cerebral cortical volume enlargement has been reported in 2- to 4-year-olds with autism. Little is known about the volume of subregions during this period of development. The amygdala is hypothesized to be abnormal in volume and related to core clinical features in autism.

**Objectives:** To examine amygdala volume at 2 years with follow-up at 4 years of age in children with autism and to explore the relationship between amygdala volume and selected behavioral features of autism.

**Design:** Longitudinal magnetic resonance imaging study.

**Setting:** University medical setting.

**Participants:** Fifty autistic and 33 control (11 developmentally delayed, 22 typically developing) children between 18 and 35 months (2 years) of age followed up at 42 to 59 months (4 years) of age.

**Main Outcome Measures:** Amygdala volumes in re-

lation to joint attention ability measured with a new observational coding system, the Social Orienting Continuum and Response Scale; group comparisons including total tissue volume, sex, IQ, and age as covariates.

**Results:** Amygdala enlargement was observed in subjects with autism at both 2 and 4 years of age. Significant change over time in volume was observed, although the rate of change did not differ between groups. Amygdala volume was associated with joint attention ability at age 4 years in subjects with autism.

**Conclusions:** The amygdala is enlarged in autism relative to controls by age 2 years but shows no relative increase in magnitude between 2 and 4 years of age. A significant association between amygdala volume and joint attention suggests that alterations to this structure may be linked to a core deficit of autism.

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**A**UTISM IS A COMPLEX neurodevelopmental disorder likely involving multiple brain systems. Converging evidence from magnetic resonance (MR) imaging, head circumference, and postmortem studies suggests that brain volume enlargement is a characteristic feature of autism,<sup>1</sup> with its onset most likely occurring in the latter part of the first year of life.<sup>2</sup> On the basis of functional MR imaging data identifying decreased amygdala activity during gaze processing,<sup>3</sup> Baron-Cohen et al<sup>4</sup> first proposed that amygdala dysfunction may account for core social characteristics of autism. Neuropathological and structural MR imaging studies have also highlighted alterations within the amygdala. Postmortem studies of individuals with autism have noted immature-appearing and densely packed cells<sup>5,6</sup> and fewer neurons within the amygdala.<sup>7</sup> Abnormal

amygdala volumes have been observed across multiple structural MR imaging studies of adolescents and adults with autism.<sup>8-13</sup> Altered amygdala activation in response to facial and emotion processing tasks also has been reported in functional MR imaging studies of individuals with autism.<sup>14-16</sup> Abnormal activation patterns were not evident in functional neuroimaging studies of individuals with autism presented with nonfacial social processing paradigms.<sup>17-20</sup> Taken together, studies of the amygdala in autism suggest that both the morphologic characteristics and function of this structure are abnormal and that amygdala dysfunction may be associated with social deficits involving facial processing.

Adolphs et al<sup>21</sup> observed deficits in recognition of negative facial emotions among patients with bilateral focal lesions of the amygdala. The authors reported that these deficits were the consequence of a failure

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**Table 1. Sample Characteristics**

Variable	Subject Group, Mean (SD)			
	Autism	Control		
		Total	DD	TYP
Time 1	(n = 50)	(n = 33)	(n = 11)	(n = 22)
Age, y	2.68 (0.32)	2.58 (0.55)	2.83 (0.42)	2.46 (0.53)
Mullen composite IQ <sup>a</sup>	53.78 (9.02)	89.21 (27.40)	56.58 (16.94)	105.82 (15.98)
Mullen AE/age	0.55 (0.17)	0.95 (0.31)	0.53 (0.27)	1.11 (0.22)
Sex, No. (%) male	43 (86)	24 (73)	10 (91)	16 (73)
Time 2	(n = 31)	(n = 20)	(n = 6)	(n = 14)
Age, y	5.01 (0.42)	4.70 (0.47)	4.97 (0.49)	4.59 (0.53)
Mullen composite IQ <sup>a</sup>	56.58 (16.94)	95.32 (28.40)	56.00 (6.77)	112.31 (12.34)
Mullen AE/age	0.53 (0.27)	0.94 (0.34)	0.61 (0.17)	1.10 (0.20)
Sex, No. (%) male	28 (90)	14 (70)	3 (50)	11 (79)

Abbreviations: AE, age equivalent; DD, developmentally delayed; TYP, typically developing.

<sup>a</sup>Mullen Scales of Early Learning composite IQ standard score.

to orient to the eye region when viewing faces. Sasson et al<sup>22</sup> recently reported that, on an emotion recognition task sensitive to deficits related to amygdala damage,<sup>23</sup> individuals with autism show decreased attention to face regions relative to age- and IQ-matched healthy control subjects and age-matched individuals with schizophrenia. Individuals with autism in this study, in contrast to healthy controls and individuals with schizophrenia, did not modulate their attention to social scenes according to whether faces were present or absent. Failure to orient to faces and, more specifically, the eye region of the face is inherent in multiple aspects of social impairment unique to autism (eg, joint attention [JA], facial emotion processing) and may be linked to amygdala abnormalities. Multiple studies have identified JA deficits as the most reliable marker of autism in the first 2 years of life<sup>24-31</sup> and thus suggest that the amygdala plays a key role in neurodevelopmental alterations unique to autism.

Both increased<sup>12,13</sup> and decreased<sup>8-11</sup> amygdala volumes have been noted in structural MR imaging studies of individuals with autism. Schumann et al<sup>12</sup> first suggested that inconsistencies across studies are the result of age-dependent effects. Observing amygdala enlargement in school-aged (7½-12½ years) but not adolescent (12¾-18½ years) autistic children, the authors hypothesized that enlargement of the amygdala in autism is an early-occurring phenomenon. Consistent with this report, Sparks et al<sup>13</sup> reported amygdala enlargement in 3- to 4-year-olds with autism, whereas studies of adolescents and adults with autism have highlighted reduced amygdala volumes compared with typically developing individuals.<sup>8,10</sup> None of these studies has observed individuals over time. Giedd et al<sup>32</sup> previously showed that longitudinal studies are necessary for characterizing neuroanatomical development within the context of interindividual variability and nonlinear growth. Studies of amygdala growth in young children with autism observed over time are needed to map developmental patterns and brain-behavior relationships unique to this disorder.

Two recent studies reported that amygdala volume is associated with social deficits in autism. Examining the

sample from the Sparks et al<sup>13</sup> study, Munson et al<sup>33</sup> reported that right amygdala enlargement at age 3 to 4 years is associated with worse concomitant social functioning and is predictive of worse social functioning at age 6 years. However, that study did not specify the aspects of social impairment in autism associated with amygdala volume. Nacewicz et al<sup>9</sup> reported that amygdala volumes were reduced in a small group of adolescents with autism (N=21) and that decreased amygdala volume was significantly associated with decreased amount of time spent fixating on the eye region of faces.<sup>9</sup>

We examined amygdala volumes and growth in children with autism at 2 years of age (the earliest age of generally accepted diagnosis) with follow-up at 4 years of age. We developed an observational coding system to examine JA and its relationship to amygdala volume. Joint attention was targeted because (1) it consistently has been shown to be impaired in young children with autism<sup>24-31</sup> and (2) as measured herein, it requires attention to the eye region of the face. Amygdala volumes were hypothesized to be enlarged in autism and significantly associated with JA deficits that involve orienting to the eye region but would not be associated with other social behaviors not involving orienting to the eye region (eg, nonverbal gesture). Amygdala volumes were also examined in relation to nonsocial characteristic features of children with autism, specifically restricted and repetitive behaviors.

## METHODS

### SAMPLE

Fifty children with autism entered this MR imaging study at 2 years (18-35 months) of age, 31 of whom were followed up at approximately 4 years of age (42-59 months of age; **Table 1**). Thirty-three control subjects (22 typically developing [TYP] children and 11 nonautistic, developmentally delayed [DD] children) entered the study at 2 years of age. The sample of control children was enriched with DD children to more closely match the autism study group in terms of IQ. The TYP and DD children were not analyzed separately because of the small

sample sizes. Twenty control subjects (14 TYP, 6 DD) were followed up at 4 years of age. Further details of subject ascertainment are reported elsewhere.<sup>2</sup> In an earlier analysis of this sample performed at age 2 years,<sup>34</sup> we observed a mean developmental quotient of approximately 54 for children with autism. We hypothesized that our ascertainment of very young children with autism may have been biased toward identifying individuals with more severe presentations and/or lower IQ that led to their detection at age 2 years. We therefore began to enrich this sample with high-functioning autistic subjects at age 4 years. At the time of the present analysis, 2 high-functioning autistic subjects had been added to the sample, for a total of 52 autistic subjects entering this study.

Subjects with autism were referred after receiving a clinical diagnosis or while on a waiting list for a clinical evaluation of autistic disorder. Subjects with DD were included only if they had no identifiable cause for their delay (eg, prematurity, genetic or neurologic disorder) and no evidence of a pervasive developmental disorder after being screened with the Childhood Autism Rating Scale. The DD and TYP children were excluded if they had Childhood Autism Rating Scale scores of 30 or greater. Medical records were also reviewed and DD subjects were excluded for evidence of autism, pervasive developmental disorder not otherwise specified, or the previously mentioned medical conditions. A standardized neurodevelopmental examination was administered to exclude subjects with any notable dysmorphic characteristics, evidence of neurocutaneous abnormalities, or other significant neurologic abnormalities. Subjects were excluded if they had evidence of a medical condition thought to be associated with autism,<sup>35</sup> including fragile X syndrome or tuberous sclerosis. Cytogenetic or molecular testing was used to rule out fragile X syndrome in autistic and DD subjects. Subjects also were excluded if they had evidence of gross central nervous system injury (eg, cerebral palsy, significant complications or perinatal/postnatal trauma, drug exposure), seizures, or significant motor or sensory impairment. Study approval was obtained from both the University of North Carolina and Duke institutional review boards, and written informed consent (from parents) was secured.

## CLINICAL ASSESSMENT

Children between 18 and 35 months of age (time 1) were eligible for inclusion in this study. Medical records were reviewed. Diagnosis was confirmed by the Autism Diagnostic Interview–Revised.<sup>36</sup> Subjects were included in the autism group if they met the interview's algorithm criteria, had scores on the Autism Diagnostic Observation Schedule (ADOS)<sup>37</sup> consistent with autism, and met *DSM-IV* criteria for autistic disorder. The diagnosis was reassessed at age 42 to 59 months (time 2), and 3 subjects who no longer met criteria for autistic disorder based on the foregoing diagnostic criteria were not included in the final analyses.

All subjects were assessed on the Mullen Scales of Early Learning<sup>38</sup> and the Vineland Adaptive Behavior Scales.<sup>39</sup> The Repetitive Behavior Scale–Revised<sup>40</sup> also was administered to assess 6 domains of repetitive behaviors: stereotypical behaviors (ie, purposeless movements that are repeated in a similar manner), self-injurious behaviors (ie, behaviors that cause physical self-harm and are repeated), compulsive behaviors (ie, behaviors that are repeated according to a rule), ritualistic behaviors (ie, activities of daily living repeated in a similar manner), sameness behavior (ie, resistance to change), and restricted behavior (ie, limited range of focus, interest, or activity). The Repetitive Behavior Scale–Revised was examined in relation to amygdala volume to assess the specificity of hypothesized relationships between the amygdala and JA.

We developed a measure of social orienting, the Social Orienting Continuum and Response Scale (SOC-RS),<sup>41</sup> that has been previously validated and that incorporates both dimensional and categorical response codes. The SOC-RS ratings are applied during observation of videotaped ADOS sessions. Ratings are performed for social orienting and communication behaviors including initiating JA (IJA), responding to JA (RJA), and nonverbal gestures.

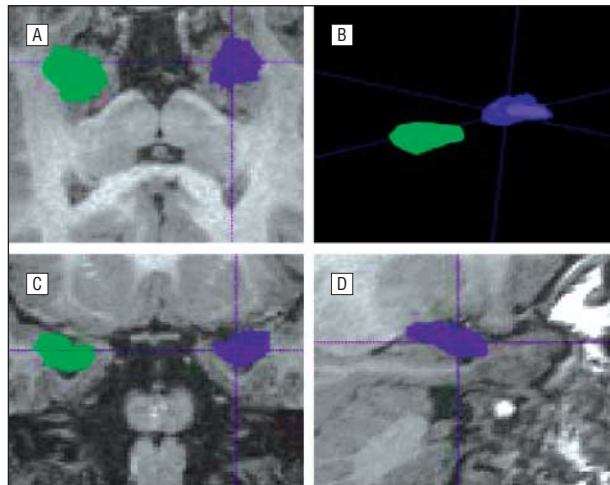
After initial analyses of behavioral data, key items were selected for analysis with the present MR imaging data. Joint attention was examined in this study on the basis of its dependence on attention to the eye region of the face. Joint attention was defined as an event in which children either initiate directing another person's attention toward an object through the use of eye gaze (ie, IJA) or follow someone else's attention toward an object by following a shift in eye gaze (ie, RJA). The JA variables, therefore, assess children's abilities when communicating attention by focusing on another individual's eyes or responding to cues specifically offered by eye movements from others. In contrast to other behaviors scored within the SOC-RS, JA requires that children attend to the eye region and process shifts in eye gaze. An IJA is scored if children start a JA and refer to the face of their social partner to monitor that person's attention (ie, if children point to an object but do not look at the examiner, then the event is not scored). An RJA is scored if children follow a shift in eye gaze by the examiner during the scheduled JA press of the ADOS. Children were not included in analyses of RJA if this activity was not observed on camera. If children did not respond to one of 5 trials, then they were scored as nonresponders. Although children were given the opportunity to merely follow a pointing gesture by the examiner during the ADOS, only events in which children followed a shift in eye gaze were scored because of our interest in children's ability to process information from the eyes. A JA total (JAT) score was computed by assigning a score of 1 for children who scored greater than 0 on either the IJA or RJA variable. The rate of nonverbal communicative gestures not involving attention to the eye region (pointing, clapping) was also examined as a control variable to investigate the specificity of relationships between amygdala volume and JA variables. Gesture rate scores were calculated by dividing the frequency by total observed time.

To eliminate bias from inadequate sampling, children were not included in analyses if they were not observable on camera for at least 10 minutes. This minimum time limit was set to allow for a representative sample of behavior. Because final analyses compared only rates (frequency/time) of behaviors and responses that were presented to each individual over a fixed number of trials, duration of observable behavior should not affect results.

To establish reliability of SOC-RS items, raters independently coded 15 videotaped ADOS sessions 2 times. Reliability was calculated by means of intraclass correlation coefficients. After establishing an intrarater and interrater reliability score of greater than 0.8 across these 15 cases, each rater independently coded cases for final analysis. Good interrater reliability was observed for IJA (0.80), RJA (0.86), and gestures (0.81).

## MR IMAGE ACQUISITION

All subjects underwent imaging on a 1.5-T MR imaging scanner (Signa; General Electric Co, Milwaukee, Wisconsin) at the Duke–University of North Carolina Brain Imaging and Analysis Center located at Duke University Medical Center. Image acquisition was designed to maximize gray/white tissue contrast at age 2 to 4 years and included the following: (1) a coro-



**Figure 1.** Sample segmentation of right and left amygdala using ITK-SNAP software. Visualization includes axial plane (A), volumetric representation (B), coronal plane (C), and sagittal plane (D). Image is presented in radiologic orientation with the right hemisphere visualized on the left side of the image (green) and the left hemisphere on the right side (blue).

nal T1 inversion recovery prepared: inversion time, 300 milliseconds; repetition time, 12 milliseconds; echo time, 5 milliseconds; 20° flip angle; 1.5-mm thickness with 1 excitation; 20-cm field of view; and 256 × 192 matrix and (2) a coronal proton density/T2 two-dimensional multislice dual echo fast spin echo: repetition time, 7200 milliseconds; echo time, 17/75 milliseconds; 3.0-mm thickness with 1 excitation; 20-cm field of view; and 256 × 160 matrix. A series of localizer images and a set of phantoms were used to standardize assessments over time and across individuals.

At both time points, in preparation for imaging, subjects with autism and subjects with DD received moderate sedation (combination of pentobarbital sodium and fentanyl citrate as per hospital sedation protocol) administered by a nurse and under the supervision of a pediatric anesthesiologist. A more detailed description appears elsewhere.<sup>42</sup> At age 2 years, TYP subjects underwent imaging without sedation, in the evening, during natural sleep. At age 4 years, a subset of 5 TYP subjects were trained to lie still in a practice scanner by means of behavioral techniques, including desensitization and positive reinforcement; children viewed videos of their choice and the video remained on while children remained still.<sup>43</sup> Parents of TYP children identified whether they wished their children to participate with or without the behavioral training. All images were reviewed by a pediatric neuroradiologist for significant clinical abnormalities. No evidence of qualitative neuroanatomic abnormalities was observed for any of the children included in the present study, on the basis of a clinical review by a neuroradiologist.

### IMAGE PROCESSING

A standardized tracing protocol was used for the amygdala and briefly is described as follows. Reliability was obtained by 2 raters who made independent measurements on a set of 15 images, which included 5 images repeated 3 times (in random order). The amygdala was manually traced on high-resolution T1 images aligned along the long axis of the hippocampus by means of the ITK-SNAP tool<sup>44</sup> following a protocol developed by the Center for Neuroscience and the M.I.N.D. Institute at the University of California, Davis.<sup>12</sup> We first established reliability with the M.I.N.D. Institute group (average interrater reliability, 0.90) on adult subjects. Subsequently, reliability was established on images from our sample of 18- to 35-month-olds. Average in-

trarater reliability was  $r=0.93$ , and interrater reliability was  $r=0.90$ . A single rater ( $r=0.90$ ) performed all amygdala traces. See **Figure 1** for an example of amygdala tracing.

### STATISTICAL ANALYSES

Group differences were evaluated for age, sex, and developmental IQ. As expected, given the disproportionate rate of males with autism, sex was unequally distributed across groups. Sex also is known to be associated with brain volume and, therefore, was included as a covariate in all analyses. An insufficient number of females with autism was available to perform separate analyses by sex. Given the variability of age across subjects (18-35 months), age was also included as a covariate. The IQ of the autistic group was significantly lower than that of the controls; therefore, IQ was included in the analyses as a covariate.

The first set of analyses examined group differences in amygdala volumes and growth rates. A series of mixed models with repeated measures of the amygdala volume domains (hemisphere, time) were fit with amygdala volume as the dependent variable, diagnostic group as the predictor of interest, and age, sex, and IQ as covariates. This resulted in up to 4 observations per subject (left and right amygdala, times 1 and 2). Diagnostic group was entered as a 2-level categorical variable (autism, controls). Estimates for the controls were created by using postestimation procedures to combine the group estimates. Age, sex, IQ, and group were included as predictors in each of the models, along with all 2- and 3-way interactions with hemisphere (right or left) and group. The significance of the interactions with hemisphere was examined. If none of these interactions was significant, then the effects were reported as averages across the left and right amygdala. To evaluate whether the group difference was proportional to that observed in total tissue volume (TTV), a second model was fit that added TTV and the 2-way interaction with age. The TTV included all cortical, subcortical, and brainstem gray and white matter and was selected rather than total brain volume because it provides a more specific index of brain enlargement without inclusion of increases due to ventricular enlargement. Results using total brain volume were not different than those using TTV for the analyses.

Relationships between JA and amygdala volume were examined by adding the SOC-RS JA ratings and interaction terms to the models described previously. The IJA, RJA, JAT, and gestures were examined separately. Two-tailed tests were conducted for the amygdala-behavioral analyses.

## RESULTS

### AMYGDALA VOLUME

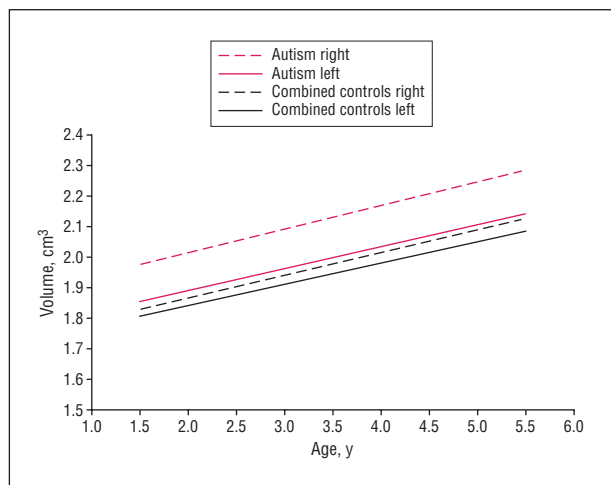
Because of insufficient image quality or artifact, we were unable to adequately visualize the amygdala in 8 subjects (5 with autism, 1 TYP, and 2 DD). The ratio of images that were of insufficient quality did not differ between diagnostic groups.

Significant hemisphere effects were observed across groups for amygdala volumes ( $F_{2,85}=3.14$ ,  $P=.048$ ). Right amygdala volumes were larger than left amygdala volumes. Therefore, analyses are reported separately for the right and left amygdala.

### CHANGE OVER TIME

Amygdala volume increased significantly over time in the total sample of autistic and control subjects ( $\beta=0.14$ ,  $SE=0.02$ ,  $P<.001$ ) (**Figure 2**). The slope of amygdala





**Figure 2.** Mean right and left amygdala growth trajectories for the autism and control groups adjusted for age, IQ, and total tissue volume. Both groups show significant growth, but the rate of change does not differ between groups.

**Table 2. Group Means and Differences in Amygdala Volume Adjusted for Age, Sex, and IQ**

	Autism vs Controls		
	Difference, Mean (SE), mm <sup>3</sup>	P Value	Percentage Difference
Time 1			
Total	0.324 (0.085)	.03	20
Right	0.368 (0.086)	<.001	23
Left	0.279 (0.088)	<.001	18
Time 2			
Total	0.249 (0.102)	.02	13
Right	0.293 (0.105)	.006	15
Left	0.204 (0.104)	.05	11
Main effect			
Total	0.295 (0.085)	<.001	17
Right	0.339 (0.087)	<.002	19
Left	0.250 (0.087)	.005	15

growth remained positive and significant after adjusting for TTV ( $\beta=0.07$ ,  $SE=0.02$ ,  $P=.002$ ). Group comparisons of change in amygdala volume over time were not significant before or after controlling for TTV.

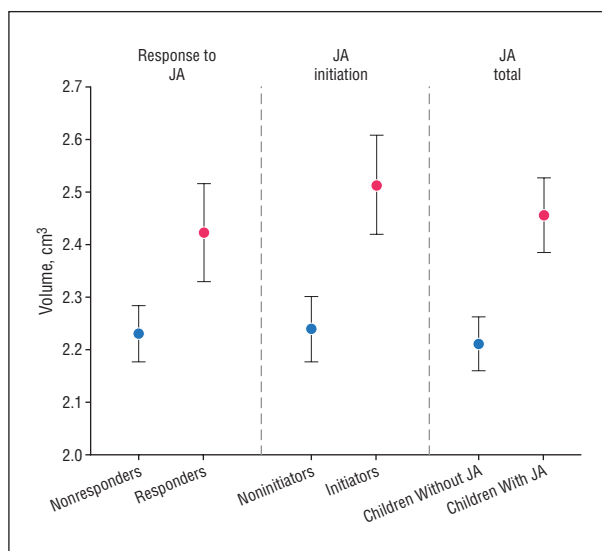
Because no group differences were observed in rate of amygdala volume change over time, amygdala volumes at times 1 and 2 were averaged (**Table 2** and **Table 3**). When average amygdala volumes were compared, individuals with autism had significantly larger right and left amygdala volumes than controls. After controlling for TTV, only the right amygdala remained enlarged in the autism group relative to the control group. Results did not change when the 2 high-functioning children with autism were excluded from analyses, nor did they change when females were excluded.

#### AMYGDALA AND JA

Clinical correlates of amygdala volume were examined for the autism group only; ADOS (and, consequently, SOC-RS) data were not available for controls. At time 1,

**Table 3. Group Means and Differences in Amygdala Volume Adjusted for Age, Sex, IQ, and Total Tissue Volume**

	Autism vs Controls		
	Difference, Mean (SE), mm <sup>3</sup>	P Value	Percentage Difference
Time 1			
Total	0.090 (0.073)	.22	5
Right	0.135 (0.076)	.08	7
Left	0.046 (0.076)	.55	2
Time 2			
Total	0.104 (0.084)	.22	5
Right	0.149 (0.088)	.10	7
Left	0.060 (0.084)	.48	3
Main effect			
Total	0.096 (0.065)	.15	5
Right	0.140 (0.069)	.045	7
Left	0.051 (0.067)	.45	3



**Figure 3.** Mean and standard error of amygdala volumes (average of right and left amygdala) contrasted for children with autism who did and did not engage in joint attention (JA). Data are adjusted for age, IQ, and sex.

8 of 39 children with autism (21%) initiated and/or responded to JA (ie, a JAT score of 1); 9 of 23 children (39%) initiated and/or responded to JA at time 2. At time 1, 7 of the 39 autistic children (18%) initiated JA; 9 of 23 children (39%) initiated JA at time 2. At time 1, 7 of 39 children with autism (18%) responded to JA (ie, shifts in eye gaze) bids from the examiner; at time 2, 4 of 21 children (19%) responded to JA initiated by the examiner. At time 1, 19 of 39 children (49%) made at least 1 nonverbal communicative gesture; 16 of 24 (67%) gestured at time 2.

The relationship between amygdala volume and JA indexes did not differ when right and left amygdala volume were analyzed separately; therefore, right and left volumes were averaged and combined for analysis. A significant positive association was observed at age 4 years between amygdala volume and JAT (**Figure 3**;  $\beta=0.25$ ,  $SE=0.07$ ,  $P<.001$ ). This relationship was also significant for amygdala volume and IJA ( $\beta=6.04$ ,  $SE=2.04$ ,

$P = .006$ ), and between amygdala volume and RJA ( $\beta = 0.19$ ,  $SE = 0.09$ ,  $P = .04$ ). The relationship between amygdala volume and gestures was not significant for either 2- or 4-year-olds with autism. The relationships between amygdala volume and the 6 subscales of the Repetitive Behavior Scale-Revised were not significant at age 2 or 4 years. Results did not change after exclusion of females from the analyses.

## COMMENT

Comparison of amygdala volume showed bilateral enlargement in children with autism. Right amygdala volume was enlarged disproportionately to TTV increases, and left amygdala was enlarged proportionately to TTV increases. Consistent with previous research,<sup>7,33</sup> right amygdala enlargement was found to be more robust than left amygdala enlargement. Autistic subjects showed a 5% increase in TTV at ages 2 to 4 years,<sup>34</sup> whereas observed amygdala volumes were 16% larger than in the group of 2- to 4-year-old controls. Amygdala enlargement was present by age 2 years. Growth trajectories between 2 and 4 years of age did not differ in autistic children and non-autistic controls. These findings suggest that, consistent with a previous report of head circumference growth rates in autism<sup>34</sup> and studies of amygdala volume in childhood,<sup>12,13,33</sup> amygdala growth trajectories are accelerated before age 2 years in autism and remain enlarged during early childhood. Moreover, amygdala enlargement in 2-year-old children with autism is disproportionate to overall brain enlargement and remains disproportionate at age 4 years.

Amygdala enlargement in autism was associated with increased JA and, despite the findings that only the right amygdala volume was increased relative to TTV enlargement, the strength of the relationship between JA and amygdala volumes did not differ by hemisphere. It is important to note that the left amygdala is enlarged, but this enlargement is not disproportionate to TTV. These results are consistent with both previous studies establishing a significant association between amygdala volume and social functioning in autism.<sup>9,33</sup>

Amygdala enlargement was associated with JA ability at age 4 years but not with communicative gestures. Analyses performed as part of a forthcoming detailed report on basal ganglia and behavior in autism indicated that the relationship between caudate nuclei volumes and JA in this sample was not significant at either 2 or 4 years of age, suggesting that the relationship between amygdala volume and JA is specific (H.C.-H., Michael Graves, BA, R.G.-S., M.W.M., M.D.P., G.G., and J.P., unpublished data, 2008). Joint attention is distinct from other social behaviors measured in the present study because it involves social orienting and eye contact with others. Adolphs et al<sup>21</sup> postulated that damage to the amygdala limits individuals' natural tendency to orient to the eye region of faces. The observation that amygdala volume is associated with JA and not other social behaviors suggests that amygdala alterations in autism reflect diminished social orienting behavior and, more specifically, reduced tendency to coordinate eye contact. Reduced JA

engagement in autism precludes shared social experiences and thus can have a cascade of developmental effects, including disrupted cognitive, communication, and social cognitive growth.<sup>26</sup> The association between amygdala volume abnormalities and attention to eyes has now been established in 2 independent studies (the present study and that of Nacewicz et al<sup>9</sup>), suggesting that this association is evident from early childhood through adulthood.

Nacewicz et al<sup>9</sup> reported *decreased* amygdala volumes associated with *reduced* eye contact in adolescents and adults with autism. The association between amygdala *enlargement* and *increased* JA ability in autism observed herein is consistent with these findings but also suggests nonlinear growth patterns in autism.<sup>45</sup> Both Schumann and Amaral<sup>7</sup> and Nacewicz et al<sup>9</sup> hypothesize an "allostatic overload" model to explain nonlinear patterns of amygdala growth in autism. Within this model, repeated exposure to a highly stimulating event leads to a compensatory response (allostasis) within the amygdala, including increased dendritic arborization and consequent overgrowth. The compensatory response involves excess production of corticotropins and glucocorticoids that, on surpassing a threshold concentration (allostatic overload), result in cell death within the amygdala. Initial amygdala hypertrophy in autism is thus followed by reduced amygdala volume later in development. The present results indicate that amygdala enlargement emerges before age 2 years and persists, but does not increase in magnitude, between 2 and 4 years of age. This enlargement is associated with attention to eyes and, although the mechanisms linking amygdala enlargement and JA ability are not known, the present results are consistent with the hypothesis that an allostatic process in which dendritic arborization and overgrowth result from sensitivity to processing eyes is evident in autism at approximately age 4 years.

The amygdala plays a critical role in early-stage processing of facial expression<sup>19,46-48</sup> and in alerting cortical areas to the emotional significance of an event.<sup>49</sup> The amygdala, via afferent connections projecting from the superior colliculus and pulvinar nucleus of the thalamus,<sup>50</sup> alerts upstream cortical regions, including the fusiform face area of the fusiform gyrus, orbitofrontal cortex, and superior temporal sulcus, to the emotional salience of stimuli such as faces. Damage to the primate amygdala during adulthood has inconsistent effects on social interactions but, if occurring during infant development, leads to increased social fear within novel environments.<sup>51,52</sup> Amygdala disturbances early in development, therefore, disrupt the appropriate assignment of emotional significance to faces and social interaction.

Schultz<sup>53</sup> previously suggested that early amygdala alterations in autism during social processing contribute to later deficits in face processing and higher-order social cognition. He hypothesized that experience with faces in infancy corresponds with enhanced salience assigned by the amygdala, which, in turn, leads to motivation to preferentially allocate attentional resources to faces. Dawson et al<sup>26</sup> hypothesized that early social deprivation in autism resulting from a lack of social attention (and concomitant failure to promote interaction through JA) dis-

rupts normative trajectories of neural and behavioral development. The association between amygdala enlargement and JA ability observed herein thus suggests that amygdala overgrowth in autism may contribute to subsequent cortical face processing system disturbances<sup>54</sup> and core social and cognitive developments, as are evident in autism.

The primary limitation within this study was that few children with autism demonstrated JA abilities at age 2 or 4 years. The behavioral observations coded for the present study target multiple social behaviors and provide only a small number of presses for JA. Inclusion of additional attempts to elicit JA across multiple contexts may increase power to identify children with autism who engage in JA at earlier ages. An additional limitation was that the relationship between amygdala volume and JA could not be investigated in control groups. Assessing whether the pattern of amygdala-JA findings differs in autistic and nonautistic children will be important for understanding brain-behavior associations unique to autism. Last, the small number of females with autism included in our study suggests that future investigation is needed to determine whether amygdala enlargement and the observed relationship between amygdala volume and JA each are evident in females with autism.

## CONCLUSIONS

We observed bilateral amygdala enlargement in a large sample of 2-year-olds with autism that persisted through 4 years of age. This enlargement was disproportionate to TTV enlargement for the right amygdala as well. Continued follow-up (now under way) of this sample will be necessary to examine whether amygdala growth rates in autism continue to parallel those seen in nonautistic individuals, or whether a second period of accelerated growth or period of volumetric atrophy occurs in autism after age 4 years. Similarly, longitudinal MR imaging studies of high-risk neonates will provide insights into the onset of amygdala overgrowth in autism.

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