ORIGINAL ARTICLE

# Amygdalar Volume and Behavioral Development in Autism

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**Context:** The amygdala is associated with socioemotional function and has been implicated in the pathophysiology of autism.

**Objective:** To examine the relationship between amygdalar volume at ages 3 and 4 years and severity of clinical course and outcome at 6 years of age in children with autism spectrum disorder.

**Design:** Magnetic resonance images acquired at 3 and 4 years of age were used to measure total cerebral, amygdalar, and hippocampal volumes. Acquisition of social and communication skills was assessed semiannually using the Vineland Adaptive Behavior Scales. Hierarchical linear models were used to predict variability in individual linear growth trajectories as a function of IQ, total cerebral, and amygdalar or hippocampal volumes.

**Setting:** Longitudinal study of children with autism spectrum disorder.

**Participants:** Forty-five children with autism spectrum disorders between 3 and 6 years of age.

**Main Outcome Measure:** Linear growth trajectory of age equivalence Vineland communication and social scores.

**Results:** Larger right amygdalar volume was associated with more severe social and communication impairments at ages 3 and 4 years. Larger right amygdalar volume also was predictive of poorer social and communication abilities at age 6 years, even after controlling for IQ and total cerebral volume. Parallel analyses with hippocampal volumes found no relationship to social or communication development.

**Conclusions:** Larger right amygdalar volume at 3 and 4 years of age, but not left amygdalar, hippocampal, or total cerebral volume, is associated with a more severe clinical course and worse outcome at age 6 years in children with autism spectrum disorder. These results provide additional evidence that amygdalar development is implicated in the behavioral impairments found in autism.

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AUTISM IS A NEURODEVELOPMENTAL disorder characterized by impairments in social relatedness and communication skills and a restricted range of interests, including stereotyped behaviors. Autism spectrum disorder (ASD) can be reliably diagnosed by age 2 years, with some symptoms appearing by the end of the first year of life.[1] By preschool age, behavioral symptoms of autism include deficits in socially coordinated eye gaze, emotional responses, and motor imitation.[2,3] Despite recognition of the syndrome and the prediction more than half a century ago by Kanner[4] that its associated social-affective difficulties are innate and biologically driven, the neuropathologic etiology of autism remains poorly understood.

Research linking specific neuroanatomical regions with social and emotional processing in typically developing individuals has allowed for testable hypotheses regarding the neural basis for social impairments found in autism. The amygdala and related medial temporal lobe structures, in particular, have been linked to a variety of social behaviors in animal and human studies, including recognition of facial expressions, gaze, biological motion and animacy, visual detection of potentially threatening stimuli and appropriate inhibition toward them, interpretation of gaze, and social or emotional tagging of stimuli.[5-12] Several neuropathologic studies of individuals with autism have demonstrated abnormalities of the amygdala, although findings have been inconsistent.[13-20]



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Overall, the weight of evidence implicating the amygdala in autism has led to the proposal of an "amygdala theory of autism."[21]

To more fully understand the relationship between amygdalar development and the social-communicative impairments in autism, longitudinal investigations of the brain and behavioral development are necessary. Neuroimaging of young children with autism is of particular utility in understanding the pathophysiology of autism. While such studies remain uncommon because of the methodological challenges of imaging young clinical populations, understanding the developmental course of brain and behavior within individuals, at as early an age as a diagnosis can be established, can help clarify relationships between developmental abnormalities in specific brain structures and functional deficits in autism.

The data presented in the current study were collected as part of a National Institutes of Health Collaborative Programs of Excellence in Autism longitudinal study of children with ASD. Three-dimensional magnetic resonance images (MRIs) were acquired for morphometric measurements of the amygdala, as well as for other brain structures, in children with ASD, children with developmental delay, and typically developing children between the ages of 3 and 4 years. Brain structural findings for this cohort of 3- and 4-year-old children have been reported previously.[18] Findings indicated that children with ASD had larger cerebral volumes compared with typically developing children (9.8% larger) or children with developmental delay (12.5% larger). Despite prior reports of a bimodal distribution of cerebral volumes in ASD,[22-24] no evidence of a bimodal distribution of cerebral volumes was found in this sample of young children with ASD. As well, cerebral volumes were not correlated with cognitive ability at ages 3 and 4 years. From our prior work, an unexpected finding was that the children with ASD had increased amygdalar volumes bilaterally relative to typically developing children. This amygdalar enlargement remained significant for a more severely affected subgroup of children with ASD diagnosed with autistic disorder (AD) compared with less severely affected children with pervasive developmental disorder not otherwise specified (PDD-NOS), even after controlling for larger total cerebral volume.

It is well recognized that individuals with ASD have tremendous variability in outcome, yet little is known about the factors accounting for these individual differences. The current study, therefore, extends our prior work assessing brain structural differences at 3 and 4 years of age by exploring the predictive relationships between early amygdalar volume at ages 3 and 4 years and the rate of acquisition of social and communicative skills and outcome through 6 years of age in this same cohort of children with ASD. Given the potential role of the amygdala in autistic symptom expression and the finding in the present sample of enlarged amygdalae at 3 and 4 years of age, we were interested specifically in investigating whether early large amygdalar volume was predictive of a slower acquisition of social and communication skills and poorer outcome by age 6 years.

## METHODS

### PARTICIPANTS

The current study included 45 children with ASD (38 boys, 7 girls). Sex distribution of the sample was representative of the general ASD population. Participants' chronological ages ranged from 38 to 54 months at the time of their MRI, with a mean ± SD age of 47.4 ± 4.2 months. Children were recruited in the greater Seattle, Wash, area from parent advocacy groups, preschools, the Washington State Department of Developmental Disabilities, clinics, and hospitals. Children with significant motor or sensory impairment, major physical abnormalities, seizures, a history of serious head injury, an identifiable neurological disorder, prenatal or perinatal difficulties, metal implants, or those regularly taking psychoactive medications were excluded. The parent or legal guardian of each participating child provided written informed consent in compliance with the University of Washington internal review board.

The current study was conducted with a previously reported sample of children with ASD, further divided into clinical subgroups of AD and PDD-NOS based on symptom severity.[18] In this group, 29 children met criteria for AD (3 girls, 26 boys; mean ± SD age, 46.9 ± 4.3 months [range, 38-54 months]) and 16 children met criteria for PDD-NOS (4 girls, 12 boys; mean ± SD age, 48.2 ± 4.0 months [range, 42-54 months]). To establish a diagnosis of AD or PDD-NOS, trained health care professionals at the University of Washington Autism Center administered the Autism Diagnostic Interview–Revised (ADI-R)[25] and the Autism Diagnostic Observation Schedule–Generic (ADOS-G)[26] and made a clinical judgment of diagnosis based on criteria defined in *DSM-IV*.[27] Final diagnostic classification was based on the integration of the clinical evaluation and the 2 standardized diagnostic instruments. Children included in the group with AD met criteria for autism on the ADOS-G and ADI-R (or within 2 points of criteria) and *DSM-IV* criteria for autistic disorder. Children were included in the PDD-NOS group if they met autism criteria on the ADI-R but PDD-NOS on the ADOS-G and by *DSM-IV* clinical criteria.

Cognitive ability was measured using the Mullen Scales of Early Learning,[28] a well-validated, normalized measure of language, visual spatial, and motor abilities. At the time of their MRI, the mean ± SD Mullen Composite Age Equivalent Score was 25.9 ± 9.2 months. A composite ratio IQ was calculated by taking the age equivalent score divided by the child's chronological age.

### MRI PROCEDURE

The protocol for MRI data acquisition and processing is described in detail by Sparks and colleagues.[18] Scans were performed at the University of Washington Diagnostic Imaging Sciences Research Center on a 1.5-T General Electric Signa Scanner (General Electric Medical Systems, Milwaukee, Wis), using a 3-dimensional spoiled gradient-recalled echo pulse sequence for image acquisition. The repetition time was 33 milliseconds, echo time was minimum, flip angle was 30°, the field of view was 22 cm, and a 256 × 256 matrix was used. To improve resolution during acquisition, slice thickness was reduced from 3 to 1.5 mm by zero filling in the third-phase encoding direction. The MRIs were acquired under continuous intravenous infusion of propofol at 180 to 220 μg/kg, as described elsewhere,[29] which allowed excellent image quality to be obtained, with only 2 children with ASD having suboptimal image studies for morphometric assessment.[18] Volumetric measurements were performed by a blinded rater using a semiautomated imaging analysis program (MEASURE) developed at Johns Hopkins University.[30] MEASURE allows the rater



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**Table 1. Pearson Correlations Between Brain Volume and Sex, IQ, and Autism Symptoms at 3 Years of Age in 45 Children**

| | Volume | | | | |
|---|---|---|---|---|---|
| | Total Cerebral | R Amygdalar | L Amygdalar | R Hippocampal | L Hippocampal |
| Sex (male = 0, female = 1) | −0.26 | −0.15 | −0.16 | −0.25 | −0.14 |
| Mullen Scales of Early Learning,[28] composite IQ score | 0.27 | 0.01 | −0.03 | 0.08 | 0.06 |
| ADOS-G[26] social score | −0.03 | 0.06 | −0.03 | 0 | 0.02 |
| ADOS-G communication score | 0.14 | 0.27 | 0.12 | −0.02 | 0.08 |
| ADI-R[25] social score | −0.22 | 0.16 | −0.08 | 0.05 | 0.08 |
| ADI-R communication score | 0.12 | 0.38* | 0.09 | 0.20 | 0.05 |
| ADI-R repetitive score | −0.17 | 0.05 | 0.16 | −0.09 | −0.12 |

Abbreviations: ADI-R, Autism Diagnostic Interview–Revised; ADOS-G, Autism Diagnostic Observation Schedule–Generic.
*$P = .01$.

to simultaneously visualize and interact with the region of interest within multiple dimensional planes. Neuroanatomical definitions for amygdalar and hippocampal measurements were based on Honeycutt et al,[31] as described in detail and illustrated on the Johns Hopkins Pediatric Neuroimaging Web site (http://pni.med.jhu.edu/methods/manual.htm). All volumetric measurements were performed by a single rater blinded to the subject identity and diagnosis; intrarater and interrater reliability for volumetric measurements of the total cerebrum, left amygdala, and left hippocampus have been previously reported.[18] Similar intrarater and interrater reliability for defining the right amygdala (0.97 and 0.90, respectively) and right hippocampus (0.96 and 0.94, respectively) was found using intraclass correlations to assess measurement consistency.

The cerebrum was measured using a 3-dimensional stereotaxic grid with $17 \times 17 \times 17$ points. Cerebral volumes included gray and white tissue, but not cerebrospinal or other nonbrain tissue, and included the basal ganglia and corpus callosum while excluding the ventricles, brainstem, and cerebellum.

Amygdalar volumes were measured bilaterally in the axial orientation (after reformatting from the 3-dimensional coronal MRIs) and tracing was confirmed in the sagittal and coronal views. Landmarks were the tubera where the optic nerve was separated from the mamillary body (superior border), the vertical line from the extreme edge of the most medial white matter protruding into the amygdaline gray matter (lateral), the uncus (medial), and the temporal horn of the lateral ventricles in the superior slices and at the head of the hippocampi for the inferior slices (posterior). When confirming the boundaries, any tissue anterior to the anterior commissure or medial to the uncal notch was excluded.

Hippocampal volumes served as a comparison region to the amygdalae. The hippocampi were traced in the coronal view starting posteriorly where the regional boundaries were unambiguous and then confirmed in the sagittal and axial views. The boundaries were the choroid fissure and inferior horn of the lateral ventricle (superior), the inferior temporal horn of the lateral ventricle or temporal stem white matter (lateral), the parahippocampal gyrus (inferior), and the angle where the hippocampus curved inferior medially into the parahippocampal gyrus (mesial). Any tissue lateral and superior to the parahippocampal gyrus was also included, while medial or inferior tissue was excluded. The alveus and subiculum were included, whereas the uncus and fornix were excluded.

### ASSESSMENT OF ACQUISITION OF SOCIAL AND COMMUNICATION SKILLS DURING PRESCHOOL PERIOD

The Vineland Adaptive Behavior Scales[32] socialization and communication domains were administered to each child's primary caregiver semiannually from ages 3 to 6 years. The Vineland Scales are a well-normed measure of abilities from birth to adulthood. The children in the sample had a mean ± SD 6.6 ± 1.4 (range, 3-9) Vineland assessments between the ages of 36 and 78 months. Age equivalence scores were used, which provide a measurement of the child's actual level of functioning that is independent of the child's age. Thus, change over time can be explicitly modeled within each individual child.

### STATISTICAL PROCEDURE

Hierarchical linear models[33] were used to predict variability in the individual linear growth trajectories of Vineland socialization and communication scores as a function of IQ, total cerebral volume and right and left amygdalar volume, or right and left hippocampal volume measured at ages 3 and 4 years. In contrast to multivariate repeated-measures approaches, this approach explicitly models change within the individual while simultaneously testing to what degree other factors correlate with the variability in growth across individuals.[33] A linear function, with the intercept placed at 6 years of age, was fit for each child's Vineland scores, resulting in 2 random-effect parameters (intercept or the child's outcome at 6 years of age and slope or rate of change across the time span) that describe the course of development in social and communication ability as measured by the Vineland Scales. Modeling the intercept at age 6 years provides a direct statistical test of the accumulated effect of individual differences in growth rates at age 6 years. We examined the degree to which IQ, total cerebral volume, right and left amygdalar volumes, and hippocampal volumes predicted the variability in these 2 growth parameters.

## RESULTS

### CONCURRENT RELATIONSHIPS BETWEEN BRAIN VOLUME AND AUTISM SYMPTOMS AT AGES 3 AND 4 YEARS

Zero-order correlations between total cerebral, amygdalar, and hippocampal volume and autism symptoms (ADOS-G social and communication algorithm scores; ADI-R social, communication, and repetitive algorithm scores) are presented in **Table 1**. Partial correlations between amygdalar and hippocampal volumes and autism symptoms controlling for total cerebral volume were also run. Larger right amygdalar volume was significantly correlated with greater social impairment on the ADI-R



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($r$=0.31; $P$<.05) and greater communication impairment on the ADI-R ($r$=0.37; $P$<.05) because higher scores on the ADI-R reflect endorsement of more autism symptoms. No significant relationships were observed between left amygdalar, right hippocampal, or left hippocampal volumes and these measures ($r$=–0.11 to 0.22; all $P$≥.15). When controlling for both total cerebral volume and composite IQ, right amygdalar volume continued to correlate with the ADI-R communication score ($r$=0.39; $P$=.01), while the correlation with the ADI-R social score remained similar in magnitude but fell just short of significance ($r$=0.29; $P$=.06), with no other significant relationships emerging. The cross-sectional relationships at ages 3 and 4 years between right and left amygdalar volume and ADI-R communication scores are shown in **Figure 1**.

## SOCIAL AND COMMUNICATION GROWTH AS A FUNCTION OF AGE

The first model examines the mean growth trajectory for the sample to determine whether meaningful individual variability occurred across children. These results indicate that at 6 years of age (72 months) the sample as a whole had mean±SE socialization age equivalence scores of 33.8±2.2 months and mean±SE communication age equivalence scores of 40±3.0 months. The mean±SE rate for acquisition of social skills was 0.55±0.06 month per chronological month and for communication skills, 0.72±0.07 month on average. Thus, children acquired language skills at a faster rate than social skills.

The reliability estimates provided by hierarchical linear models indicated that a substantial proportion of the observed variability in these growth parameters was true score or parameter variance that is potentially predictable by other variables. Reliabilities were as follows: socialization intercept=0.93, socialization slope=0.69, communication intercept=0.98, and communication slope=0.84.

## IQ, TOTAL CEREBRAL VOLUME, AND AMYGDALAR VOLUME AS PREDICTORS OF GROWTH

Composite IQ, total cerebral volume, and right and left amygdalar volumes were entered as level 2 predictors in hierarchical linear models. All variables were standardized prior to analysis to create equivalent metrics among the predictors. IQ emerged as the strongest predictor of rate of acquisition (slope) and outcome (intercept at age 6 years) for social and communication skills, whereas total cerebral volume was not related to these measures of social and communication growth (**Table 2**). Larger right amygdalar volume was associated with poorer social and communicative outcome at age 6 years, even after controlling for IQ, total cerebral volume, and left amygdalar volume (Table 2). Conversely, after controlling for IQ, total cerebral volume, and right amygdalar volume, smaller left amygdalar volume was associated with worse communicative outcome at age 6 years. Variation in growth trajectories for social skills and communication development in relationship to right amygdalar volume are illustrated in **Figure 2**.

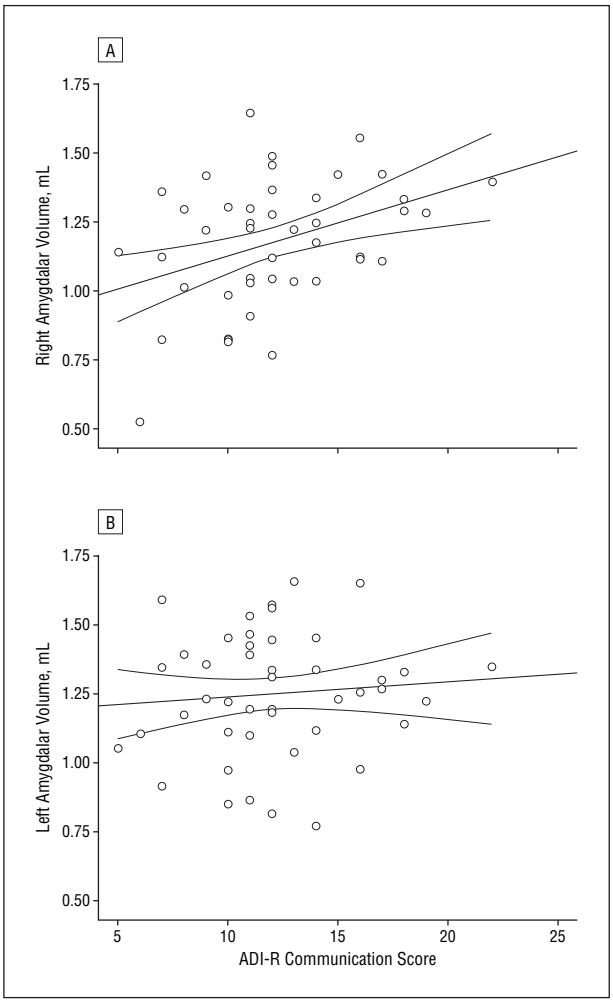


**Figure 1.** Scatterplot between concurrent Autism Diagnostic Interview–Revised[25] (ADI-R) communication score and right (A) and left (B) amygdalar volume.

To address the question of specificity, parallel analyses were conducted using right and left hippocampal volumes. No relationships between right or left hippocampal volumes and children's social and communication development or outcome at age 6 years were observed (Table 2).

## COMMENT

We previously reported that compared with age-matched children with developmental delay and typical development, 3- and 4-year-old children with ASD demonstrated larger than expected total cerebral volume as well as proportionally larger amygdalar volumes.[18] For a subgroup of children with AD who were more severely affected, there was disproportionate bilateral amygdalar enlargement beyond what would be expected after controlling for larger total cerebral volume. While most previous studies have focused on volumetric or morphological differences between diagnostic groups, this study, using prospective longitudinal behavioral data, provides evidence that larger right amygdalar volume at 3 and 4 years of age is associated with slower acquisition



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**Table 2. Hierarchical Linear Model Results Predicting Variability in Vineland Socialization and Communication Growth From 3 to 6 Years of Age***

| | Vineland Socialization Score | | | Vineland Communication Score | | |
|---|---|---|---|---|---|---|
| | Coefficient | *t* Test | *P* Value | Coefficient | *t* Test | *P* Value |
| Amygdala | | | | | | |
| Outcome at age 6 y (intercept) | | | | | | |
| Constant | 33.80 | 19.80 | <.001 | 40.10 | 21.23 | <.001 |
| IQ | 7.55 | 4.59 | <.001 | 14.76 | 8.67 | <.001 |
| Right amygdalar volume | −6.19 | −2.24 | .03 | −5.64 | −2.34 | .02 |
| Left amygdalar volume | 3.66 | 1.52 | .14 | 5.52 | 2.13 | .04 |
| Total cerebral volume | 1.95 | 0.76 | .45 | 0.11 | 0.05 | .96 |
| Rate of change between ages 3 and 6 y (slope) | | | | | | |
| Constant | 0.55 | 9.97 | <.001 | 0.72 | 11.72 | <.001 |
| IQ | 0.18 | 3.64 | .001 | 0.25 | 4.31 | <.001 |
| Right amygdalar volume | −0.13 | −1.55 | .13 | −0.15 | −1.99 | .05 |
| Left amygdalar volume | 0.08 | 0.96 | .34 | 0.12 | 1.45 | .16 |
| Total cerebral volume | 0.04 | 0.47 | .64 | 0.02 | 0.27 | .79 |
| Hippocampus | | | | | | |
| Outcome at age 6 y (intercept) | | | | | | |
| Constant | 33.83 | 17.33 | <.001 | 40.14 | 20.26 | <.001 |
| IQ | 5.73 | 2.60 | .01 | 14.61 | 8.87 | <.001 |
| Right hippocampal volume | 0.63 | 0.25 | .80 | −0.45 | −0.15 | .88 |
| Left hippocampal volume | −1.68 | −0.62 | .54 | −1.51 | −0.57 | .57 |
| Total cerebral volume | 0.82 | 0.34 | .73 | −0.30 | −0.14 | .89 |
| Rate of change between ages 3 and 6 y (slope) | | | | | | |
| Constant | 0.55 | 9.45 | <.001 | 0.72 | 11.56 | <.001 |
| IQ | 0.14 | 2.36 | .02 | 0.25 | 4.47 | <.001 |
| Right hippocampal volume | −0.08 | −0.97 | .34 | −0.10 | −1.05 | .30 |
| Left hippocampal volume | 0.05 | 0.54 | .59 | 0.03 | 0.36 | .74 |
| Total cerebral volume | 0.03 | 0.36 | .72 | 0.02 | 0.22 | .83 |

*Vineland Adaptive Behavior Scales.[32]

of social and communicative skills and poorer outcome at 6 years of age within a sample of children with ASD.

This association between enlarged right amygdalar volume and poorer socialization and communication development and outcome persisted even after controlling for IQ and total cerebral volume. Although a child's IQ at age 3 years was strongly predictive of his or her social and communicative growth during the next 3 years, right amygdalar volume served to independently predict additional variability in outcome at age 6 years. Findings specifically implicating enlargement of the right amygdala in impaired social and communicative behavioral development had not been predicted a priori, particularly because both right and left amygdalar volumes had been found to be enlarged in this ASD sample.[18] Although the basis for the association between enlarged right amygdalar volume and poorer social and communication development remains to be understood, recent evidence from healthy controls suggests differential right-sided amygdalar activation in response to conditioned fear and supports that there may be lateralization of emotional response patterns mediated by the amygdala.[34] The specificity of the relationship between right amygdalar enlargement and poorer outcome is also supported by a failure to find a similar relationship for the left amygdala. In fact, larger left amygdalar size at ages 3 and 4 years predicted improved language outcome at age 6 years.

The observed relationship between individual differences in amygdalar size and variations in social and communication outcome adds to previous literature suggesting that the amygdala is abnormal in autism. Furthermore, these findings might help to resolve conflicting findings across studies regarding abnormal amygdalar histopathological and morphological abnormalities and volume in autism. For example, some post-mortem findings have suggested reduced neuronal size and increased packing density in the amygdala of adults with autism, as well as abnormal neuronal histopathological abnormalities in a variety of related regions,[13,35] while several groups have not identified neuronal or morphological abnormalities in the amygdala.[36-38] Neuroimaging studies also provide accumulating, yet also inconsistent, evidence for abnormal amygdalar structure in autism. Some groups have reported no differences in amygdalar size between individuals with autism and controls,[14] while others have found reduced size[15-17] or increased size.[18-20] Most of these studies, however, have not directly linked volumetric findings to specific behavioral correlates. Often studies have grouped participants of wide age ranges and symptom severity. Differences in findings might reflect differing methods, including neuroimaging parameters for data acquisition, definitions of the region of interest, or criteria used for clinical diagnosis. Yet, these factors may not account for all the inconsistencies reported. For example, good measurement reliability was established between Aylward et al[16] and Sparks et al[18] in tracing the amygdalae, yet the findings of their studies differed and were postulated to reflect different age ranges and symptom se-



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verity of participants studied (ie, adolescents and adults with autism compared with 3- to 4-year-old participants with ASDs). Therefore, careful attention to the developmental course and diagnostic subgroups within the autism spectrum as well as careful comparison of imaging methods appear to be important considerations in understanding the role of individual differences in brain structure, such as differences in amygdalar volume. Prospective longitudinal techniques offer a way of determining the impact of structure on function as well as the developmental course, allowing for more definitive conclusions regarding causality.

Longitudinal work may also be useful in determining causality and tracking individual change, building on work such as that described in this study relating structural abnormalities of the amygdalae and functional deficits more directly in individuals with ASDs. Group differences between individuals with high-functioning autism and typical adolescents and adults on recognition of fearful faces, eye gaze direction detection, and facial recognition memory were inversely related to bilateral amygdalar volumes, suggesting increased amygdalar volume might be linked to impairments in social perception.[39] Using functional neuroimaging, several differences in amygdalar activation to social stimuli have been found. Baron-Cohen and colleagues[40] reported reduced activation in the left amygdala to a theory of mind task compared with controls, while Critchley and colleagues[41] found less activation in the left amygdala–hippocampal region of individuals with autism than controls during implicit processing of facial expressions.

Future longitudinal work must be informed by efforts across a variety of disciplines to gain understanding of amygdalar function, by testing relationships between amygdalar structure with increasingly specific behavioral measures over time. Existing research encompassing a variety of converging approaches such as neuropsychological investigation of individuals with brain damage, primate lesion studies, and functional neuroimaging of both typical and atypical populations has sought to link amygdalar function to impairments in social and emotional functioning. Evidence from structural damage to the amygdalae in animals and humans suggests a variety of related social impairments that may be born out in individuals with autism due to abnormal development of the amygdale. For example, Kluver-Bucy syndrome, which results from bilateral amygdalar and inferior temporal cortical damage, is associated with abnormal responses to emotional stimuli (eg, in primates, approaching stimuli that produce characteristic fear responses, no "chattering," and impaired facial expression; in humans, inappropriate sexual behavior and loss of appropriate fear and anger responses) and impaired emotional learning.[42] Hetzler and Griffin[43] have described the similarities between Kluver-Bucy syndrome and autism. Zola-Morgan and colleagues[44] found emotional impairments but not memory loss with isolated amygdalar damage, reporting dissociable functions of the amygdala and hippocampus. Impaired recognition of emotional information from faces has also been found in otherwise typically developing individuals with amygdalar damage[45] as well as in individuals with autism.[46] Such behavioral markers as production and understanding of facial expression, social approach and avoidance, and emotional responses are candidate behaviors for longitudinal study.

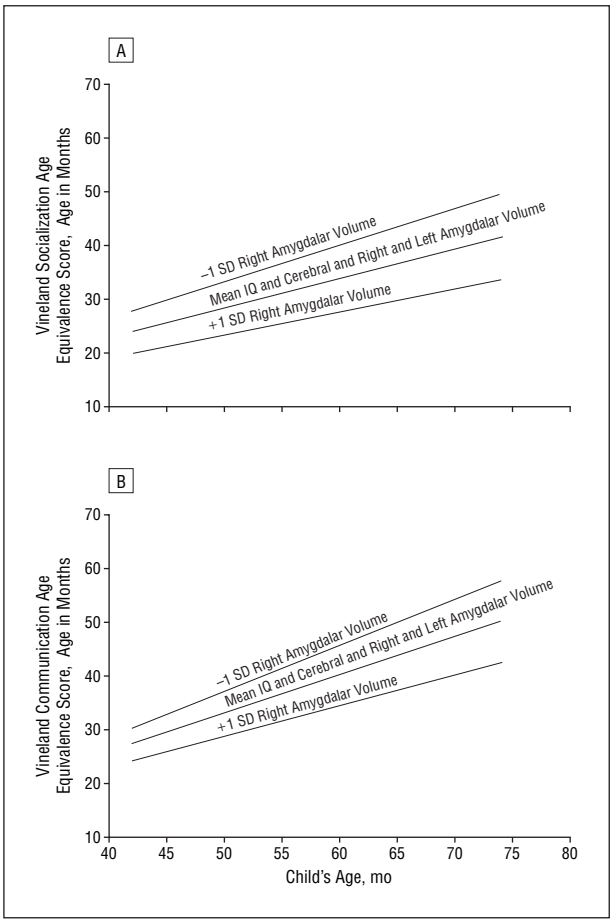


**Figure 2.** Estimated Vineland Adaptive Behavior Scales[32] growth trajectories as a function of right amygdalar volume. A, Vineland socialization domain. B, Vineland communication domain.

In more recent primate work, the developmental course of amygdalar damage has been systematically assessed by lesioning juvenile animals, providing particularly provocative clues that have particular utility in understanding the development of amygdalar function. Bachevalier [47,48] compared lesions with either the medial temporal lobe (including the amygdala, periamygdaloid cortex, hippocampus, and entorhinal and perirhinal cortex) with lesions of only the hippocampus and amygdala in rhesus monkeys at age 2 or 6 months. Results suggest that earlier medial temporal lobe lesions produced more passivity, increased temper tantrums, and reduced initiation of social contact, while the lesions of older animals led to behaviors such as active withdrawal from social approaches, emotionally blank faces, reduced eye contact, more independent activity, and stereotyped motor behaviors. Lesions limited to the amygdala alone resulted in similar, but reduced, social deficits and did not eliminate facial expressions or produce motor stereotypy.

While these more recent studies offer compelling parallels between lesions of young monkeys and the potential social deficits produced by an abnormally develop-



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ing amygdala in autism, they are not without criticism. For instance, Amaral and colleagues[49] have suggested that the lesions result in damage to pathways connecting to other regions and that postsurgical data consisted primarily of qualitative or anecdotal behavioral descriptions. To substantiate this position, Amaral et al bilaterally lesioned the amygdalae using ibotenic acid (described in Emery et al[10]) and used computerized observations to code primate social behaviors cataloged by Capitanio and colleagues[50,51] in adult and 2-week-old macaque monkeys. In the group that was lesioned after brain maturation, more affiliative social behavior, less social inhibition (ie, lesioned monkeys did not seem to evaluate other monkeys before interactions), and "attraction" to the lesioned monkeys by control animals was observed. However, these monkeys did not show social impairments apart from disinhibition and reduced ability to evaluate threat. They interpreted and generated gestures and initiated and received affiliative behaviors. When monkeys were lesioned prior to brain maturation (ie, at age 2 weeks), they interacted similarly with their mothers as controls. As adults, like adult-lesioned monkeys, they showed less fear of normally fear-producing objects (eg, rubber snakes), but unlike adult-lesioned monkeys, they exhibited increased fear response (ie, fear grimaces, screams) during dyadic social interactions. They were otherwise similar to control animals in social interaction demonstrating similar grooming, play, and facial expressivity. These findings suggest a more nuanced view of amygdalar function and indicate that the amygdala may modulate social cognition by detecting threat. It is possible that disruption of the amygdala may result in dysregulated connectivity, which in turn could inhibit or disinhibit social behavior.

Importantly, the findings of Amaral et al highlight the importance of understanding the developmental sequelae of early amygdalar damage. Machado and Bachevalier[52] also emphasize the importance of understanding the effects of early lesions via longitudinal studies in their thorough review of primate models of childhood psychopathology, suggesting early focal damage to medial temporal lobe structures may lead to a broad range of disorganization in other regions as the brain reorganizes following insult. These investigators also hypothesized that reorganization may vary with the nature, timing, and scope of the initial insult.

In recent years, progress has been made in identifying the biological substrates of the social, emotional, and communication impairments associated with autism. The current study adds an additional dimension to this investigation—time. By following the development of a structure implicated in autism, the amygdala, and tracking development in 2 key domains of impairment, social and communication, the interrelationship between brain and behavior may more clearly be understood. In continued work at the University of Washington, repeated MRI have and are currently being collected from the same group of children, enabling tracking of structural and volumetric changes within individual children. Further, the identification of amygdalar size as a marker of later development in very young children has led to the addition of amygdalar volume as a predictor of response to early intervention in a randomized study of early intensive behavioral intervention in toddlers with autism. The identification of biological markers of symptoms is significant in classifying the endophenotype of autism and ultimately of practical utility in improving clinical diagnostic power, predictive specificity in matching individual characteristics to treatment, and uncovering etiology potentially leading to prevention. Finally, additional work tracing the trajectory of other aspects of brain development, such as unusual morphological abnormalities, and other structures, such as the surrounding medial temporal lobe, in conjunction with behavioral development may prove fruitful in future work with autism.

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