Associations between the size of the amygdala in infancy and language abilities during the preschool years in normally developing children

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ARTICLE INFO

Article history:
Received 20 July 2009
Revised 11 September 2009
Accepted 12 October 2009
Available online 10 October 2009

Keywords:
Amygdala
Infant
Language development
Naturally sleeping MRI
Emotion

ABSTRACT

Recently, structural MRI studies in children have been used to examine relations between brain volume and behavioral measures. However, most of these studies have been done in children older than 2 years of age. Obtaining volumetric measures in infants is considerably more difficult, as structures are less well defined and largely unmyelinated, making segmentation challenging. Moreover, it is still unclear whether individual anatomic variation across development, in healthy, normally developing infants, is reflected in the configuration and function of the mature brain and, as importantly, whether variation in infant brain structure might be related to later cognitive and linguistic abilities. In this longitudinal study, using T1 structural MRI, we identified links between amygdala volume in normally developing, naturally sleeping, 6-month infants and their subsequent language abilities at 2, 3 and 4 years. The images were processed and manually segmented using Cardviews to extract volumetric measures. Intra-rater reliability for repeated segmentation was 87.73% of common voxel agreement. Standardized language assessments were administered at 6 and 12 months and at 2, 3 and 4 years. Significant and consistent correlations were found between amygdala size and language abilities. Children with larger right amygdalae at 6 months had lower scores on expressive and receptive language measures at 2, 3, and 4 years. Associations between amygdala size and language outcomes have been reported in children with autism. The findings presented here extend this association to normally developing children, supporting the idea that the amygdalae might play an important but as yet unspecified role in mediating language acquisition.

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Introduction

Over the last decades, structural MRI studies in children have increasingly been used to ascertain relations between brain volume and various behavioral measures (Reiss et al., 1996; Mosconi et al., 2009). Studies comparing normally developing children to children with developmental disorders such as autism (Schumann et al., 2004; Mosconi et al., 2009), Attention Deficit Hyperactivity Disorder (Castellanos et al., 2002), Down’s Syndrome (Pinter et al., 2001), Specific Language Impairment (Gauger et al., 1997), as well as children with Generalized Anxiety Disorder (De Bellis et al., 2000) and children born prematurely (Peterson et al., 2000; Zacharia et al., 2006), have identified differences in the size of various brain structures and, in some cases, have reported associations with cognitive and language measures (Reiss et al., 1996; Peterson et al., 2000; Munson et al., 2006; Mosconi et al., 2009). Nevertheless, most of these studies have been conducted in children older than 2 years of age and only a few structural MRI studies have examined normal brain development between birth and age 2 (but see Matsuzawa et al., 2001; Nishida et al., 2006; Gilmore et al., 2007; Knickmeyer et al., 2008). In one of these studies, regional neonatal brain growth was investigated in a group of 12 neonates with gestational ages ranging from 31.1 to 42.6 weeks (Nishida et al., 2006). However, none of these studies of infants younger than age 2 have examined amygdala volumes. Giedd et al. measured the volume of the temporal lobe, superior temporal gyrus, amygdala, and hippocampus in children 4–18 years old and reported gender differences in the pattern of growth of the amygdala and hippocampus (Giedd et al., 1996). Likewise, Caviness et al. reported specific patterns of brain growth and variation in brain structures in 7–11 year-old children (Caviness et al., 1996a). There is also evidence from child and adolescent samples that much can be learned by examining the impact of early variations in the trajectories of anatomical brain development on later outcomes (Lenroot and Giedd, 2006; Shaw et al., 2006). However, relatively little is known about the significance of normal variation in brain structure across infancy, including volumetric increases and decreases.
at various time points as well as changes in laterality. Furthermore, how individual anatomic variation in the normally developing infant brain might relate to the configuration and function of the more mature brain and to later cognitive and language abilities is largely unknown and unstudied.

The amygdala, often referred to as the amygdaloid complex, is a structurally and functionally heterogeneous region comprised of a group of nuclei located in the anterior temporal lobe of the cerebral hemispheres (Swanson and Petrovich, 1998). The development of the amygdaloid region is still quite poorly understood (Swanson, 2003). It is an important part of the limbic system to which it has widespread connections. Its two main connecting tracts are the stria terminalis, which is adjacent to the lateral ventricle and the ventral amygdalofugal pathway that lies in the substantia innominata in the basal forebrain. Further, the amygdala is intimately connected with the hypothalamus and has dense projections to the brainstem, the frontal and temporal lobes, and to the thalamus (Swanson and Petrovich, 1998). The amygdala has been strongly implicated in the processing of emotion including implicit emotional learning and memory (Phelps and LeDoux, 2005a; Rodrigues et al., 2004). To date, the majority of amygdala research has focused on processing of stimuli that evoke negative emotions, such as fear (Fanselow and Gale, 2003; Phelps et al., 2001). A few more recent studies, however, have reported amygdala activation in response to positive stimuli (Hamann and Mao, 2002). Moreover, there is great interest in understanding potential functional differences in the role of the left as compared to the right amygdala in emotional processing (Baas et al., 2004; Markowitsch, 1998). For instance, Hamann and Mao reported greater left amygdala activation to both positive and negative emotional words as compared to neutral words (Hamann and Mao, 2002). The interactions recently reported between emotion and cognition (Wolfe and Bell, 2007) have made it increasingly evident that the amygdala may well play a broader role in modulating cognitive function than was previously thought (Phelps, 2004, 2005). There are also indications that the amygdala’s role in memory enhancement and consolidation has been underestimated. Specifically, perceptual information reaches the amygdala through connections with both cortical and subcortical brain areas, (Phelps, 2004, 2005; Stein et al., 2007), and at this point in the processing stream, the amygdala can enhance episodic memory for the “essence of an emotional event” at the expense of details (Adolphs et al., 2001; Phelps, 2004). Further, the amygdala is thought to modulate both the encoding and subsequent storage of hippocampal-dependent memories (Phelps, 2005).

In autistic populations, for both children and adults, associations have been reported between the size of the amygdala and language measures. For example, Haznedar, et al. found that adults with Asperger's Disorder had larger left amygdalae at 3–4 years of age in children with autism was associated with more severe social and communication impairment at 3–4 years as well as at 6 years of age; smaller left amygdalae at 6 years were also associated with poorer communicative outcome. Conversely, larger left amygdala at 3–4 years predicted better language outcome at 6 years of age (Monson et al., 2006). These studies support the idea of functional lateralization of the amygdala (larger left amygdala, better language abilities and larger right amygdala, poorer language abilities) while also suggesting that the amygdala might play a role in language processing in subjects with autism.

Given such reports of associations between amygdala volume and language outcome in individuals with autism, and the fact that amygdala size and associated language abilities have not as yet been examined in normally developing infants and children, a detailed examination of the relationship between morphometric and behavioral measures and possible mechanisms underlying such associations seemed warranted. Although obtaining volumetric measures in infants is considerably more difficult as gray/white borders and discrete brain structures are less well defined (Kennedy et al., 2002), we were able in a preliminary report, with a smaller sample, to describe a significant pattern of associations between larger left amygdalae at 6 months and higher expressive language scores at 24 months (Ortiz-Mantilla et al., 2007). The present study expands on those preliminary findings and further examines relations between amygdala volume at 6 months and language outcomes through age 4 years. Given that the amygdalae are involved in the modulation of emotional memories that are decoded, consolidated and stored in the hippocampus, and that it is likely that those processes are enhanced by arousal and attention, we hypothesized that the amygdala could play a role in early language acquisition due to the fact that babies begin to decode, consolidate and store speech information while learning within the highly emotional context that characterizes the infant–parent interaction. Thus, the aims of this study were to investigate volumetric brain structure, specifically, the volume of the amygdala, in normally developing infants at 6 and 12 months and determine the pattern of volumetric changes across time. Further, we were interested in examining the associations between the size of the amygdala in infancy and the language abilities of these children as they matured from 6 months to 4 years of age. We also explore potential mechanisms by which the amygdala at early ages might be associated with later language outcomes.

Methods

Participants

The study sample was a subset of a larger study sample of normally developing children that received structural scans using a naturally sleeping MRI technique (Paterson et al., 2004). The aim of the larger study was to establish developmental landmarks, including normative anatomic baselines and variation in brain development across the first 2 years of life. In the present study, twenty-seven full-term (≥37 weeks gestational age and birth weight >2500 g) normally developing children were recruited from urban and suburban communities in New Jersey. All children (15 boys and 12 girls) were born healthy, full-term (38 to 42 weeks of gestation; mean = 40.35 weeks, SD = 8.9) and ranged in weight from 2886 to 4649 g (mean = 3577 g, SD = 433). All infants had uneventful prenatal and perinatal circumstances and were born into English monolingual families without a history of language impairment, Autism Spectrum Disorders, congenital hearing loss or other neurological or psychiatric disorders. There was no history of hearing dysfunction or recurrent episodes of otitis media.

Socio-demographic data, as well as information about infant and maternal health and obstetrical history were collected at the 6-month visit via parental questionnaire. In addition, follow-up questionnaires solicited additional infant health data at each subsequent visit. The socioeconomic status (SES) was assessed by the Hollingshead Four Factor Index (Hollingshead, 1975) and was not related to cognitive or language abilities at any of the ages tested. Infants came from families classified as middle-to upper-middle class (SES ranging from 32 to 66, mean = 55.75, SD: 8.9). Informed consent approved by the Rutgers Human Subjects Board was obtained from all parents prior to their child’s inclusion in the study that was conducted according to the principles expressed in the Declaration of Helsinki. Parents were compensated for their time and babies received a toy after their visit.

Procedure

Children visited the laboratory at 6 and 12 months, and also at 2, 3, and 4 years. At each age a comprehensive battery of age-appropriate perceptual, cognitive and language tests were administered over the
course of three to four visits. Assessments were conducted by psychologists, speech language pathologists, and highly trained research assistants. At 6 and 12 months, one of the visits was scheduled for late afternoon/early evening so that a non-sedated naturally sleeping MRI could be more easily acquired. In the imaging suite an attempt was made to replicate normal bedtime routines for the child by including soft lullaby music, a rocking chair, a crib, and any other objects or materials that might encourage sleeping (for a detailed explanation of the scanning procedures, please see Liu et al., 2008; Paterson et al., 2004). T1-weighted 3D SPGR images were collected on a GE 1.5 T Echospeed MRI scanner using a standard head coil and with the following parameters: Field of view = 25 cm, TR/TE = 24/10 ms, flip angle = 30°, matrix size = 256 × 192, slice thickness = 1.5 mm, number of slices = 124, sagittal orientation, bandwidth = 15.63 KHz. The images were then positionally normalized, and processed in Cardview, a highly detailed neuroanatomical knowledge-based, semi-automated brain segmentation tool, using previously described highly reliable and reproducible segmentation methods, and labeled to extract volumetric data (Caviness et al., 1996a,b,c; Filipek et al., 1989, 1991, 1992, 1994; Kennedy, 1986; Kennedy and Nelson, 1987; Kennedy et al., 1989, 1994; Makris et al., 1999; Meyer et al., 1999; Rademacher et al., 1992). For the purposes of this paper, we focus on volumes from the amygdalae (total, left and right) at 6 and 12 months of age. The anatomical borders of the amygdala were defined according to Filipek et al. (1994) as follows: “superiorly by the interface with the ansa peduncularis, medially by the CSF (cortico-cerebrospinal) interface, inferolaterally by white matter, and inferomedially by a secondary border connecting the medial tip of the subjacent white matter with the uncal notch” (Figs. 1a, b). In adult brain, typically only coronal and sagittal views are used for amygdala segmentation. However, for this study of infant brain the contrast in coronal and sagittal views was poorer than expected, so a reference to the axial view was used as well. A single researcher (MSC) blind to subject segmented the amygdalae twice for all participants. The intra-rater reliability for amygdala segmentation was 87.73 PCV (percentage of common voxel agreement) obtained following the method described by Caviness et al. (1996c). Intra-rater agreement was judged “very good” and was comparable to that reported in a previous study of infant brain volume (Nishida et al., 2006).

**Standardized measures**

**Cognitive assessments**

To assure that all children had normal cognitive development, two measures were administered as follows:

*The Mullen Scales of Early Learning* (Mullen, 1995) at 6 and 12 months and again at 2 years of age to assess cognitive abilities in infancy. The Mullen consists of five scales: Gross Motor, Visual Reception, Fine Motor, Receptive Language and Expressive Language that have normative scores and also gives a single composite score that represents general intelligence. Each of the Mullen scales yields a T-score (mean of 50 standard deviation of 10) and the Mullen composite score yields a standardized score (mean = 100, SD = 15).

*The Stanford–Binet Intelligence Scale–4th ed.* (SB-4) (Thordike et al., 1986) was administered at 3 and 4 years of age, to assess cognitive ability during the preschool ages. The SB-4 explores four areas of cognitive abilities: Verbal Reasoning, Abstract/Visual Reasoning, Quantitative Reasoning, and Short-Term Memory. In addition to scores for each area that are reported as Area scores, a composite standard score representing general reasoning ability (mean = 100, SD = 16) was generated.

**Language measures**

*The MacArthur Communicative Development Inventories (CDI)* (Fenson et al., 1993) was completed at 12 and 24 months. The CDI is a parental questionnaire that provides a valid and efficient means of assessing communicative gestures and play, early imitation, language comprehension, language production, and the early stages of grammatical development. At 12 months, parents completed the infant version (Words and Gestures) and at 2 years, the toddler version (Words and Sentences) that primarily measures language expression. The CDI provides age- and gender-normed percentile scores that have been shown to be stable over time, as well as highly correlated with other language and communication measures (Fenson et al., 1994).

*The Preschool Language Scale–4th ed.* (PLS-4) (Zimmerman et al., 2002) assesses receptive (Auditory Comprehension) and expressive (Expressive Communication) language skills in children from birth to 6 years and 11 months of age and was administered at 6 and 12 months, and at 2, 3, and 4 years. The test yields standard scores (mean = 100, SD = 15), percentile ranks, and age scores for the subscales as well as a total language score. For this study, standard scores for the Auditory Comprehension and Expressive Communication subscales were used.

*The Clinical Evaluation of Language Fundamentals—Preschool* (CELF-P) (Wiig et al., 1992) was administered at 3 and 4 years of age. Children were evaluated with two subtests of the CELF-P (mean = 10 SD = 3): Sentence Structure (SS) and Word Structure (WS). The Sentence Structure subtest evaluates comprehension of sentence formation rules (receptive language), while the Word Structure subtest evaluates the child’s use of morphological rules and forms (expressive language).
Results

Standardized cognitive and language measures

Cognitive ability

All children in this study had cognitive abilities that fell within the normal range. The Mullen total standard score for the sample had a mean of 96.63 (SD = 8.6) at 6 months, a mean of 104.23 (SD = 13.5) at 12 months and a mean of 113.3 (SD = 16.5) at 2 years of age. Repeated-measures ANOVA, conducted to investigate whether there were differences between the Mullen total score at the three ages, showed a significant increase in the total score from 6 to 24 months (F[2,36] = 10.21, p <.001). At later ages, the Stanford–Binet Intelligence Scale was used to measure cognitive skills. The mean full IQ composite Score at 3 years was 110.2 (SD = 10.6) and 109.2 (SD = 10.2) at 4 years. The difference between the two ages was not significant (t[10] = .73, p = .47).

Language ability

At 6 and 12 months and at 2, 3, and 4 years, children’s expressive and receptive language abilities were assessed by the PLS-4. Means and standard deviation for all language measures are shown in Table 1. A repeated measures ANOVA examining the effect of age on expressive language scores was not significant (F[4,48] = 1.64, p = .22). Conversely, we found significant differences as a function of age on the receptive language measure (F[4,48] = 3.44, p = .046) with higher scores observed at older ages (2, 3 and 4 years) as compared to younger ages (6 and 12 months). In addition, at 3 and 4 years of age the CELF-P was administered. Repeated-measures ANOVAs revealed no significant differences for expressive (F[1,13] = 3.27, p = .094) or receptive measures (F[1,13] = .38, p = .54) when 3 and 4 years scores were compared. At 12 months and at 2 years of age, parents completed the CDI (Words and Gestures at 12 months, and Words and Sentences at 2 years). As would be expected, significant correlations were found between concurrent language assessments with ranges between r = .60 and r = .89 at age 2; r = .65 and r = .85 at age 3, and r = .71 and r = .79 at age 4.

Volumetric measures

Twenty-four infants at 6 months (11 girls and 13 boys) and 15 infants at 12 months (6 girls and 9 boys) were successfully scanned; 12 of the children in this sample (5 girls and 7 boys) had successful MRIs at both ages. In this study we focus on total amygdala volume, and on the volume of the left and right amygdala. Amygdala volumes at 6 and 12 months for total, right and left amygdala volume are presented in Table 2a. Paired samples T-tests were conducted to investigate differences in amygdala volume from 6 to 12 months, including only the 12 children that had MRIs at both ages, as well as right/left amygdala volume proportions at each age. We found a significant increase in the total and right but not in the left amygdala volume from 6 to 12 months (Table 2b). No differences were seen between the mean volume of the left and the right amygdala at 6 months (t[23] = −1.39, p = .178) or at 12 months (t[14] = 1.59, p = .133). No significant differences in cognitive (F[1,17] = 2.29, p = .149) and language (expressive: F[1,17] = 1.16, p = .296; receptive: F[1,17] = 1.108, p = .307) measures at 12 months were identified between children that had MRIs at both ages and those who only had MRIs at 6 months.

Symmetry index

Proportions of right/left amygdala were examined using a symmetry index (Galaburda et al., 1990), with the index calculated for right versus left amygdala volume, according to the formula (Left − Right) / 0.5 (Left + Right). The sign of the resulting value indicates the direction of asymmetry (positive = left side larger; negative = right side larger). At 6 months, 10 infants (5 girls/5 boys) had a right bigger than left symmetry index and 14 (6/8) had a left bigger than right symmetry index. At 12 months, 10 infants (5/5) had right bigger than left, and 5 (1/4) showed left bigger than right symmetry indexes. Pearson’s Chi Square statistic did not show any significant difference in the distribution of the symmetry index as a function of gender at 6 (χ² = .120, p = .73) or at 12 (χ² = 1.25, p = .26) months. Moreover, no gender differences were apparent for total, right and left volume of the amygdala at 6 months (t[22] = −1.84 p = .079, t[22] = −1.40

Table 1

<table>
<thead>
<tr>
<th>Language measures administered from 6 months to 4 years of age.</th>
<th>6 months</th>
<th>12 months</th>
<th>2 years</th>
<th>3 years</th>
<th>4 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preschool Language Scale 4th ed. (PLS-4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expressive score</td>
<td>102.6 (6.8)</td>
<td>104.7 (8.7)</td>
<td>105.5 (21.9)</td>
<td>115.7 (14.9)</td>
<td>107.7 (12.9)</td>
</tr>
<tr>
<td>Receptive Score</td>
<td>98.4 (8.6)</td>
<td>104.9 (9.9)</td>
<td>105.4 (15.8)</td>
<td>113.7 (8.1)</td>
<td>107.5 (15.5)</td>
</tr>
<tr>
<td>Words and sentences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sentence structure</td>
<td>9.06 (2.8)</td>
<td>11.0 (3.6)</td>
<td>9.14 (3.7)</td>
<td>10.14 (3.7)</td>
<td>9.36 (3.3)</td>
</tr>
<tr>
<td>Clinical Evaluation of Language Fundamentals—Preschool (CELF-P)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phrases</td>
<td>44.4 (26.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comprehension</td>
<td>39.9 (26.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production</td>
<td>31.6 (24.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total gestures</td>
<td>40.8 (25.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MacArthur Communicative Development Inventories (CDI) Words and gestures</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production</td>
<td>35.6 (29.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irregular words</td>
<td>40.4 (29.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complexity</td>
<td>45.0 (31.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sentence length</td>
<td>3.74 (1.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: values in the table represent mean score (standard deviation).

Table 2a

<table>
<thead>
<tr>
<th>Mean volume of the total, right and left amygdala at 6 and 12 months for all study infants.</th>
<th>6 months (n=24)</th>
<th>12 months (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amygdala</td>
<td>2.15 (.32)</td>
<td>2.44 (.34)</td>
</tr>
<tr>
<td>Right amygdala</td>
<td>1.06 (.19)</td>
<td>1.25 (.16)</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>1.10 (.17)</td>
<td>1.18 (.21)</td>
</tr>
</tbody>
</table>

Note: values in the table represent mean volume in cc (standard deviation).

Table 2b

<table>
<thead>
<tr>
<th>Mean volume of the total, right and left amygdala at 6 and 12 months for the subset of infants scanned at both ages.</th>
<th>6 months (n=12)</th>
<th>12 months (n=12)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total amygdala</td>
<td>2.11 (.33)</td>
<td>2.53 (.32)</td>
<td>−3.17</td>
<td>.009*</td>
</tr>
<tr>
<td>Right amygdala</td>
<td>1.03 (.17)</td>
<td>1.29 (.19)</td>
<td>−3.98</td>
<td>.002*</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>1.08 (.18)</td>
<td>1.24 (.20)</td>
<td>−2.01</td>
<td>.070</td>
</tr>
</tbody>
</table>

Note: values in the table represent mean volume in cc (standard deviation). The T-statistics corresponds to the pair comparisons of the amygdala volume for the 12 babies that were scanned at both, 6 and 12 months of age. * p<.005. Significant p-values are bolded.
Table 3
Associations between 6 and 12-month amygdala volume and 2–4 years language measures.

<table>
<thead>
<tr>
<th>Language measures</th>
<th>Right amygdala 6 months</th>
<th>Left amygdala 6 months</th>
<th>Right amygdala 12 months</th>
<th>Left amygdala 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 years</td>
<td>Preschool Language Scale 4th ed. expressive score</td>
<td>−.55* (.01)</td>
<td>−.36 (.12)</td>
<td>−.55 (.06)</td>
</tr>
<tr>
<td></td>
<td>Preschool Language Scale 4th ed. receptive score</td>
<td>−.51* (.03)</td>
<td>−.36 (.13)</td>
<td>−.07 (.83)</td>
</tr>
<tr>
<td></td>
<td>CDI Words and Sentences production score</td>
<td>−.51* (.04)</td>
<td>−.30 (.25)</td>
<td>−.35 (.29)</td>
</tr>
<tr>
<td></td>
<td>CDI Words and Sentences complexity score</td>
<td>−.59* (.01)</td>
<td>−.47* (.04)</td>
<td>−.42 (.20)</td>
</tr>
<tr>
<td>3 years</td>
<td>Preschool Language Scale 4th ed. expressive score</td>
<td>−.46* (.05)</td>
<td>−.33 (.17)</td>
<td>−.11 (.74)</td>
</tr>
<tr>
<td></td>
<td>Preschool Language Scale 4th ed. receptive score</td>
<td>−.55* (.02)</td>
<td>−.57* (.01)</td>
<td>−.01 (.98)</td>
</tr>
<tr>
<td></td>
<td>CELF-P word structure score</td>
<td>−.54* (.03)</td>
<td>−.42 (.09)</td>
<td>−.33 (.35)</td>
</tr>
<tr>
<td>4 years</td>
<td>Preschool Language Scale 4th ed. expressive score</td>
<td>−.61* (.02)</td>
<td>−.41 (.14)</td>
<td>−.49 (.17)</td>
</tr>
<tr>
<td></td>
<td>Preschool Language Scale 4th ed. receptive score</td>
<td>−.56* (.04)</td>
<td>−.28 (.34)</td>
<td>−.24 (.54)</td>
</tr>
<tr>
<td></td>
<td>CELF-P word structure score</td>
<td>−.54* (.04)</td>
<td>−.40 (.18)</td>
<td>−.26 (.53)</td>
</tr>
</tbody>
</table>

CDI, MacArthur Communicative Development Inventories; CELF-P, Clinical Evaluation of Language Fundamentals– Preschool; * p-value ≤ 0.05. Note: values in the table represent r-values (p-values). All significant p-values are bolded.

Associations between amygdala volume in infancy and later language abilities

Pearson’s product moment correlations were conducted to examine the associations between the volume of the amygdala in infancy (at 6 and 12 months) and language abilities during the preschool years (2, 3 and 4 years). Significant and consistent correlations were found between the volume of the right amygdala at 6 months and both expressive and receptive language measures at 2, 3 and 4 years (Table 3). Children with larger right amygdala volume at 6 months showed lower PLS-4 expressive and receptive language scores at 2, 3, and 4 years of age (Figs. 2a,b; 3a,b; and 4a,b), lower language production and complexity (expressive scores) on the CELF Words and Sentences at 2 years of age, and lower expressive scores on the CELF-P at 3 and 4 years of age. Computation of partial correlations was not indicated, as the volume of the right amygdala and total brain volume were not significantly correlated. Further, no associations were found between any of the language measures and the amygdala symmetry index described above. Interestingly, no significant correlations were found between the size of the amygdala at 12 months and any of the later language outcomes.

Discussion

The aims of this study were to (1) investigate the volume of the amygdala at 6 and 12 months and the pattern of volumetric changes in the amygdala from 6 to 12 months in a group of normally developing infants, and (2) to examine relations between infant amygdala volume and subsequent language outcomes through age 4 years. Our findings demonstrate that the total volume of the amygdala increases from 6 to 12 months. Moreover, a specific pattern of amygdala growth was observed: while the right amygdala increased its size significantly, the increase in the volume of the left amygdala did not reach significance. Most importantly, we found consistent associations between the volume of the right amygdala at 6 months and expressive and receptive language abilities at 2, 3 and 4 years of age. Children who at 6 months had larger right amygdalae showed lower language scores at all subsequent ages. We did not find any gender-related asymmetries in the size of the amygdala at either 6 or 12 months of age. Further, the relations seen were not associated with a proportional measure of growth (i.e. the symmetry index representing right versus left amygdala volume) but only with individual measures of right amygdala volume.

Structural MRI studies examining normal human development have increased our knowledge about anatomical changes in the brain from childhood to adolescence (Caviness et al., 1996a; Giedd et al., 1996; Lenroot and Giedd, 2006; Shaw et al., 2006; Matsuzawa et al., 2001; Knickmeyer et al., 2008). Similar to findings in previous reports, our results demonstrate large individual variation in amygdala size.

$p = 1.74, \ t [22] = −1.91 \ p = .07$ or at 12 months $t [13] = −4.94, \ p = .02$ respectively. As noted below, the symmetry index was not significantly associated with any language outcomes.
within and across ages (Giedd et al., 1996; Caviness et al., 1996a), and an overall increase in total volume of the amygdala from 6 to 12 months. Although studies with young adults (Filipek et al., 1994) and with normally developing children (Giedd et al., 1996) found a significant right greater than left asymmetry for the amygdala that did not change across age, we did not find any significant left/right amygdala asymmetry at 6 or at 12 months. However, at 6 months of age, we observed a tendency for infants to have a larger left as compared to right amygdala (found for 58%). By 12 months, a shift to the pattern shown by older children and adults was beginning to appear: 66% of the 12-month-old infants had a symmetry index favoring larger volumes for right as compared to left amygdalae. Likewise, we did not find any evidence in infancy of the structural and functional sexual dimorphism observed in the amygdalae in children and adults (Giedd et al., 1996; Cahill, 2006). Perhaps, the interactive effect of sex-related steroids on the ongoing development of brain structures gradually induces the gender-specific differences reported in older children and in adults (Caviness et al., 1996a; Giedd et al., 1996; Cahill, 2006). However, at 6 and 12 months of age, gender-related structural dimorphism was not apparent. The question of functional laterality at these ages cannot be addressed, as we were not able to record brain function in this study.

Recently, insights have been gained from studies of “brain and behavior relations”, which suggest that differential growth and dynamic pruning in the normally developing brain may contribute to variation in structural volumes as well as differences in cognitive outcomes (Craik, 2006; Lenroot and Giedd, 2006; Shaw et al., 2006). Interestingly, as Shaw and Giedd et al. have demonstrated in regard to relations between cortical thickness measures in early childhood and IQ measures in adolescence, structural variation at earlier time points can be more strongly associated with behavioral outcomes than later or concurrent measures of structure (Shaw et al., 2006). This mirrors the findings described here of strong associations of language in the preschool years to amygdala volume at 6 months but no significant correlations with the later 12-month volumes.

Although the pathway(s) by which language mediation can be related to amygdala volume have yet to be elucidated and could not be examined in this study, there are a few potential mechanisms that might be hypothesized. First, while amygdala research has mainly focused on its role in the processing of emotion, and more specifically on negative emotions such as fear, recent evidence has shown that in humans the amygdala plays a role in learning to associate stimuli with positive outcomes (Johnsrude et al., 2000). The amygdala has anatomical connections with many brain areas related to cognitive...
function (Stein et al., 2007). And lately, it has been suggested that the amygdala might well play a more general role in the modulation of cognitive systems that respond to emotional cues ( Cahill and McGaugh, 1998; Phelps, 2006). Connections with both cortical and subcortical brain areas allow perceptual information to reach the amygdala by different pathways and at differing speeds: the faster subcortical pathway is more often implicated in fear conditioning, while the slower cortical pathway has been related to other types of emotional learning and memory, such as those involving the hippocampus (Phelps, 2004, 2005). The amygdala facilitates the storage of memories via arousal in the hippocampus, which then appears to modulate the post-encoding consolidation process (Phelps, 2005). This could be the case during episodic memory where emotion, by modulating arousal, attention and perception, influences the encoding of the information that will be remembered (Anderson and Phelps, 2001; Anderson, 2005; Phelps, 2006).

Second, if the literature to date is carefully examined, there are also findings within this potentially “broader role” for the amygdala that suggest specific language-related mediation. Support for associations between amygdala and language come from reports of adults with diseases that compromise the amygdala, from research findings in children and adults with autism, and finally, from studies in bilingual children and adults. For example, in adults with Fronto-Temporal Lobar Degeneration (FTLD) it is common to find amygdalae atrophy. Among the FTLD clinical variants, two of them, the Semantic Dementia variant and the Progressive Non- Fluent Aphasias variant have been reported to present with major and early language deficits. Significant amygdalar atrophy, greater on the left side, has been found in the Semantic Dementia variant and, to a lesser extent, in the Progressive Non- Fluent Aphasias variant (Whitwell et al., 2005). Additional evidence can be gleaned from human studies of Klüver–Bucy Syndrome, where damage of the temporal lobe and the amygdala co-occurs with receptive aphasia combined with amnesia, alterations in emotional and sexual behaviors, and dementia (Hayman et al., 1998; Lilly et al., 1983).

As noted, studies in children and adults with autism have also shown associations between the amygdalae and language. Adults with Asperger’s Disorder (a form of the autism spectrum disorder that presents with normal to above average language abilities) were reported to have larger left amygdalae than lower-functioning adults with autism (Haznedar et al., 2000). This study suggests that a smaller left amygdala may be associated with poor language outcome in patients with autism. Munson et al. reported as well that 3–4 year-old children with autism who had larger right amygdalae had more severe social and communication impairment at 3–4 and at 6 years old; whereas those who had larger left amygdalae had better language outcomes at age 6 (Munson et al., 2006). Finally, bilateral activation of the amygdala was reported in a functional MRI study when bilingual adults named pictures in their native language as compared to naming of pictures in a second language (Hernandez, 2009). Similarly, specific activation of the right amygdala was observed when 11-year-old bilingual children were performing a single word–reading task in their native language, but not in their second language (Owens et al., 2009). As an explanation, the authors propose a link between early cognition and emotion (Wolfe and Bell, 2007), with early language acquisition involving a lower-level processing mechanism that could be more closely related to emotional valence (Hernandez, 2009). Taken together, these varied studies suggest not only functional lateralization of the amygdala and the strong likelihood that the amygdala is significantly involved in the processing of emotional valence within speech, but also provide support for the notion that the amygdala might play a meaningful role in mediating early normative language acquisition.

A third mechanism that might shed light on accumulating evidence for a broader role for the amygdala, concerns recent findings that reductions in neuronal activity of the amygdala, due to opioid release, during positive, pleasant, emotional states are associated with a larger focus of attention (Koepp et al., 2009). It is hypothesized that the amygdala may mediate the facilitation of attention with emotion (Anderson and Phelps, 2001; Phelps, 2004) and it is further suggested that arousal plays a significant role in facilitating attention (Anderson, 2005). Emotion, acting through amygdala–hippocampal connections, can influence the encoding and storage of stimuli that “have to be remembered” by modulating attention and perception (Phelps, 2004, 2006). Thus, attention may facilitate memory and memory could be influenced by emotion. Although much more research is necessary to understand such putative mechanisms, we consider that one explanation of our findings might arise from the early and powerful “en face” interactions between the young infant and primary caregivers. Infants begin to develop and learn in an environment rich in highly emotional cues. Specifically, we hypothesize that the intensely pleasurable emotional relationship that characterizes the parent–infant interaction during the first months of life may be a modulating factor that, by enhancing arousal, attention and memory, facilitates the acquisition of early language. It would not be surprising to expect the amygdala to play a role in such an emotionally important behavior.

We did not find associations between language measures and right amygdala volume at 12 months. As mentioned previously, the brain–behavior relationship is difficult to untangle in that the volume of a structure and how it maps to function does not always correspond to a specific behavioral/cognitive function (Lenroot and Giedd, 2006). As has been shown in studies of older children, structural variation at earlier time points can be more strongly associated to behavioral outcomes than concurrent structural measures (Shaw et al., 2006). We hypothesize that an additional converging mechanism might be that the amygdala’s role in modulating language is at its peak in the earliest pre-linguistic period of development, when the foundations of language are being laid down, actively supported by intense adult-infant interaction. During this period, parents expose infants to language through the use of infant-directed speech that includes exaggerated stress, intonation, rhythm, and word segmentation, while infants respond by attending closely and imitating and babbling (Fernald, 1992; Meltzoff and Brooks, 2009). Cross-linguistic studies support the notion that highly emotional infant-directed speech may stimulate innate (structural) attentional biases that support language learning (Gleitman et al., 1988). We suggest that perhaps in normal development the first 6 months of life are particularly relevant to the involvement of the amygdala in the language learning process. By 12 months, other areas of the brain may become more involved as language skills begin to focus increasingly on comprehension, word production, and grammar.

**Study limitations**

Although the size of our sample is comparable to similar studies of brain structure (e.g. Filipke et al., 1994; Nishida et al., 2006; Zacharia et al., 2006), its modest size may well limit the generalizability of the findings to larger populations. Scanning un-sedated naturally sleeping infants is quite challenging and even after successful scans are obtained, computing reliable volumetric measures in early infancy is considerably more demanding than in older children, as these structures are smaller, more highly variable, less well defined, largely unmyelinated, and thus quite difficult to segment (Kennedy et al., 2002; Lenroot and Giedd 2006; Knickmeyer et al., 2008). By using Cardviews, a semi-automated brain segmentation tool, and manually segmenting, one by one, each infant’s brain structures we were able to obtain very good intra-rater reliabilities for such a small sub-cortical structure. It is important to note that this is a unique sample and although the sample size is modest, the effects are robust, and should be of great interest to those who wish to track normal developmental variation. Another limitation of the present study is the lack of temperament and early anxiety measures. Unfortunately, the data we
have available does not allow us to address a number of important developmental issues that relate to the broader role of the amygdala during the first two years of life. Although the main focus of the present study was to explore links between variation in amygdala volume across infancy and later language, and all analyses were strongly hypothesis-driven, the data discussed in this paper was initially gathered as part of a larger study which aimed to establish developmental landmarks in early brain development—essentially normative anatomical baselines and variation. A large behavioral battery was collected in conjunction with the structural scans (over three to four sessions at each assessment period), but not every measure in every domain could be obtained due to time constraints. However, given the intriguing results of this study, and the literature showing that measures of anxiety in older children and adolescents have been associated with larger right amygdala size (De Bellis et al., 2000), we regret not having such measures and we think this potential link should certainly be explored in future studies.

Despite these limitations, we believe that the results of this study are both striking and provocative. Although it is clear that more research is critical to elucidate the mechanisms that underlie these findings, we suggest that the amygdala appears to be involved, across age, in the modulation of cognitive and linguistic systems that respond to emotional cues.

In summary, we found significant and consistent associations between amygdala size in infancy and preschool language abilities. Normally developing children with larger right amygdalae at 6 months had lower standard scores on receptive and expressive language measures at 2, 3 and 4 years of age. These findings suggest that the amygdala may be involved in the establishment of early language, not only during the pre-verbal stages of language acquisition where highly affective content characterizes the parent–child interaction, but also at later ages when more complex and structured language emerges. Further, these results suggest that even from early infancy, functional lateralization of the amygdala can be demonstrated. Since the negative association between the size of the right amygdala and social and communication abilities has already been reported in children with Autistic spectrum disorders, the results detailed here extend this association to normally developing children. Certainly these intriguing findings, along with the studies cited above, suggest that the role of the amygdala, particularly across development, is in need of further focused study that examines domains outside of traditional thinking about fear and arousal responses.

Acknowledgments

This research was supported by the Santa Fe Institute Consortium. Additional funding came from the William Randolph Hearst Fund of Harvard University, an NSF grant to A. A. B. (#SBE-0542013 to the “Temporal Dynamics of Learning Center”), and from the Elizabeth H. Solomon Center for Neurodevelopmental Research. Special thanks go to the children who participated in this study and to their families who brought their children to the lab for countless successive visits. We would also like to thank the many colleagues and lab members who contributed to the success of this research. In particular, we want to acknowledge Drs. Wen-Ching Liu, Nino Badrizde, and Sarah Paterson along with Ms. Susan Marken and Ms. Julie Morgan Byrne for their assistance in data collection as well as in the development of the naturally sleeping MRI paradigm and infant sound attenuation system.

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