Title
Functional Connectivity of the Amygdala Is Disrupted in Preschool-Aged Children With Autism Spectrum Disorder

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Objective: The objective of this study was to determine whether functional connectivity of the amygdala is altered in preschool-age children with autism spectrum disorder (ASD) and to assess the clinical relevance of observed alterations in amygdala connectivity.

Method: A resting-state functional connectivity magnetic resonance imaging study of the amygdala (and a parallel study of primary visual cortex) was conducted in 72 boys (mean age 3.5 years; n = 43 with ASD; n = 29 age-matched controls).

Results: The ASD group showed significantly weaker connectivity between the amygdala and several brain regions involved in social communication and repetitive behaviors, including bilateral medial prefrontal cortex, temporal lobes, and striatum (p < .05, corrected). Weaker connectivity between the amygdala and frontal and temporal lobes was significantly correlated with increased autism severity in the ASD group (p < .05). In a parallel analysis examining the functional connectivity of primary visual cortex, the ASD group showed significantly weaker connectivity between visual cortex and sensorimotor regions (p < .05, corrected). Weaker connectivity between visual cortex and sensorimotor regions was not correlated with core autism symptoms, but instead was correlated with increased sensory hypersensitivity in the visual/auditory domain (p < .05).

Conclusion: These findings indicate that preschool-age children with ASD have disrupted functional connectivity between the amygdala and regions of the brain important for social communication and language, which might be clinically relevant because weaker connectivity was associated with increased autism severity. Moreover, although amygdala connectivity was associated with behavioral domains that are diagnostic of ASD, altered connectivity of primary visual cortex was related to sensory hypersensitivity.

Key words: autism, functional magnetic resonance imaging, neuroimaging, amygdala, development

METHOD
Participants
Participants were recruited through the University of California (UC) Davis MIND Institute as part of the Autism Phenome Project. The sample for this study included 72 boys (ASD n = 43; typically developing controls [TD] n = 29; mean age 3.5 years). All participants were screened by a board-certified developmental behavioral pediatrician (K.A.) and were free of seizures and the use of any psychotropic or behavioral medications. Children with ASD were screened and excluded for fragile X syndrome. All children (TD controls and children with ASD) were native English speakers, ambulatory, and had no physical contraindications to MRI, vision or hearing problems, known genetic disorders and/or other neurologic conditions. For TD controls, inclusion criteria were developmental scores within 2 standard deviations on all scales of the Mullen Scales of Early Learning.20 In addition, TD children were screened and excluded for autism using the Social Communication Questionnaire (scores >11).21 This study was approved by the UC Davis institutional review board, and informed consent was obtained from the parent or guardian of each participant before imaging. Volumetric amygdala data from a subset of these participants have been reported on previously.12,22

Diagnostic assessments for children with ASD included the Autism Diagnostic Interview–Revised23 and the Autism Diagnostic Observation Schedule–Generic (ADOS).24,25 which was used to calculate the ADOS severity score, a standardized metric of quantitative ASD symptom severity that is relatively independent of age and verbal ability.26 Sensory processing was assessed using the Short Sensory Profile,27 a parent checklist measuring a child’s sensory sensitivity in several domains (including a domain for visual/auditory sensitivity), with lower scores indicating greater hypersensitivity to sensory stimuli. Measurements of cognitive ability for all participants (i.e., overall cognitive ability, verbal ability, and nonverbal ability) were derived using standard scores from the Mullen Scales of Early Learning20 and the Differential Ability Scales.28

MRI Data Acquisition
Children were scanned during natural, nocturnal sleep29 at the UC Davis Imaging Research Center on a 3-T TIM Trio MRI (Siemens, Malvern, PA) using an 8-channel head coil. A high-resolution structural scan was acquired for anatomic parcellation and overlay of statistical maps (T1-weighted 3-dimensional magnetization-prepared rapid acquisition with gradient echo [MPRAGE]; 1-mm isotropic voxels; repetition time 2,170 ms; echo time 4.86 ms; field of view 256 mm; 192 sagittal slices). For each participant, a resting-state echo planar imaging blood oxygen level dependent (EPI-BOLD) sequence was acquired containing 300 whole-brain T2*-weighted volumes (37 interleaved axial slices per volume; 4-mm slice thickness; in-plane resolution 4 mm²; repetition time 2,000 ms; echo time 27 ms; flip angle 87°; field of view 256 mm). The duration of sleep from the onset of sleep to the beginning of the resting-state BOLD scan was recorded for each participant. This measurement served as a proxy for determining sleep stage as an alternative to collecting polysomnographic data during fMRI scanning, due to concerns that simultaneous acquisition of such data would increase the likelihood of children waking up. All scans were acquired within the first hour and a half after sleep onset. Several studies have demonstrated that young children at these similar ages are reliably found to be in non-rapid eye movement sleep stage 3 within this timeframe.30,32

MRI Analyses
The left and right amygdala were manually traced on T1-weighted images based on a study-specific anatomical protocol,12 using Analyze software.33 Manual tracings from a subset of participants were derived from a previous study on amygdala volume.12,22 The resulting regions of interest (ROIs) were used as seed regions for the functional connectivity analysis. For the comparison analysis of V1, the seed ROI of V1 was anatomically parcellated using the FreeSurfer software package.34 All functional connectivity analyses were performed using the Analysis of Functional Neuroimages (AFNI; http://afni.nimh.nih.gov/afni/) and the FMRI Software Library (FSL; http://www.fmrib.ox.ac.uk/fsl/). Of the 300 time points acquired for each participant, the first 10 were discarded due to signal instability. Then, EPI-BOLD resting-state scans were pre-processed: time shifted (AFNI: 3dTshift), motion corrected (AFNI: 3dvolreg), spatially smoothed with an effective smoothness of Gaussian 6-mm full width at half maximum (AFNI: 3dmerge), bandpass filtered at 0.008 < f < 0.08 Hz,35 co-registered to the structural image (FSL: flirt). The resulting ROI masks were visually inspected (FSLView) for anatomic accuracy in every participant to ensure that that no partial voluming had occurred (i.e., voxels in the ROI mask were contained entirely within the anatomically defined ROI). Motion scrubbing (i.e., censoring) was implemented to remove single frames that exceeded frame-wise displacement greater than 0.25 mm, and not frames preceding or following, based on recommendations by Power et al.36,37 when using global signal regression.

Several regressors were included in the individual model to remove motion and nuisance artifacts: 6 motion parameters (AFNI: 3dvolreg), white matter and cerebrospinal fluid (CSF; from FSL: FAST), and temporal derivatives of each (computed using AFNI: 1d_tool.py). Given the current debate on the use of global signal regressor (GSR),36,38-40 the present analyses were conducted both with GSR (extracted using AFNI: 3dmaskave) and without GSR; the results were compared and were strikingly similar. Therefore, results with GSR are presented in the main text (for analyses without GSR, see Figures S1 and S2, available online). The nuisance regressors and their derivatives (6 motion parameters, white matter, CSF, and GSR) were extracted from the time series before bandpass filtering; then, the time series and nuisance signals were bandpass filtered and entered simultaneously into the individual model.

Mean time series of the left and right amygdala ROIs were extracted separately from each individual (AFNI: 3dmaskave) and correlated with all other voxels in the brain (AFNI: 3dDeconvolve). Single-subject-level connectivity maps for each amygdala seed were then subjected to 1-sample t tests for within-group analyses and 2-sample independent t tests for group comparison (AFNI: 3dttest+++) in standard Montreal Neurological Institute space. The uncorrected voxel-wise p-value thresholds used before family-wise error cluster-size correction was .00001 for within-group analyses and .01 for between-group analyses. To adjust for multiple comparisons, cluster size significance—2 voxels for within-group and 32 voxels for between-group comparisons—was determined by Monte Carlo alpha simulations (AFNI: 3dClustSim and identical confirmation by AlphaSim) for a corrected significance threshold of p < .05 (AFNI: 3dmerge).

RESULTS
Participant demographic, diagnostic, and behavioral measurements are presented in Table 1. There were no group...
differences in age. As expected, overall cognitive ability, verbal ability, and nonverbal ability were significantly higher in TD controls ($p < .001$).

Several important control procedures were employed to rule out artifacts that could contribute to any observed group differences in connectivity patterns. Because each participant was scanned during natural nocturnal sleep, virtually no motion artifact was found in either group (i.e., very few time points needed to be scrubbed or removed due to frame-wise displacement > 0.25 mm)\(^{36}\) and the groups did not differ in the number of time points scrubbed (Table 1). Even so, stringent motion correction procedures did not differ in the number of time points scrubbed or removed due to frame-wise displacement ($p > .68$) on different motion parameters (Table 1), including all 6 motion directions and overall motion over the entire scan.\(^{36,41}\) Thus, group differences in connectivity could not be attributed to motion artifact. Given the current debate on the use of GSR,\(^{36,38-40}\) analyses were conducted both with GSR (Figures 1 and 2) and without GSR (Figures S1 and S2), and the findings were strikingly similar. This lack of difference in the use of GSR suggests that the data acquired in this study are of such high quality (i.e., no motion) that the white matter and CSF regressors accounted for all remaining variance attributed to noise (i.e., no significant variance remained for the GSR to regress). Notably, the groups also did not differ in the duration of sleep before the resting-state scan ($p = .50$; Table 1). This suggests that the stage of sleep did not differ between the 2 groups and did not contribute to group differences in functional connectivity.

Functional Connectivity With the Amygdala

The functional connectivity maps for the left and right amygdala are shown in Figure 1 (see Tables S1 and S2 for detailed descriptions of the clusters, available online). The ASD and TD groups both exhibited within-group connectivity between the amygdala and the striatum, bilateral medial temporal lobes, posterior ventral temporal lobes, and medial prefrontal cortex (mPFC; $p < .05$, corrected; Figure 1A–B, D–E). However, direct between-group comparison showed that the ASD group had significantly weaker connectivity between the amygdala and several brain regions involved in social communication and repetitive behaviors, including the mPFC, bilateral temporal lobe, striatum, thalamus, cingulate cortex, and cerebellum ($p < .05$, corrected; Figure 1C, F).

Comparison Analysis: Functional Connectivity With Primary Visual Cortex

To evaluate whether the patterns of connectivity differences were specific to the amygdala, rather than reflecting global connectivity differences across multiple brain systems, a comparison study was performed using V1 as the seed ROI for a functional connectivity analysis. Results for the functional connectivity analysis with V1 are shown in Figure 2 (see Table S3 for detailed descriptions of the clusters, available online). Although the ASD group showed limited V1 connectivity primarily within the occipital lobe (Figure 2B), the TD group showed additional connectivity between V1 and medial prefrontal cortex (mPFC), bilateral temporal lobe, striatum, thalamus, cingulate cortex, and cerebellum ($p < .05$, corrected; Figure 2C, F).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>ASD</th>
<th>TD</th>
<th>$p$ Value</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>43</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Age [y]</td>
<td>3.5 (0.79)</td>
<td>3.6 (0.86)</td>
<td>.46</td>
</tr>
<tr>
<td>Overall cognitive ability</td>
<td>70.6 (18.4)</td>
<td>103.2 (12.0)</td>
<td>&lt;.001</td>
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<tr>
<td>Verbal ability</td>
<td>69.1 (19.6)</td>
<td>102.3 (9.7)</td>
<td>&lt;.001</td>
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<tr>
<td>Nonverbal ability</td>
<td>72.1 (19.2)</td>
<td>104.1 (16.6)</td>
<td>&lt;.001</td>
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<tr>
<td>ADOS severity</td>
<td>8.0 (1.36)</td>
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<td>N/A</td>
</tr>
<tr>
<td>Short sensory profile</td>
<td>17.72 (5.09)</td>
<td>19.21 (3.47)</td>
<td>.36</td>
</tr>
<tr>
<td>Motion parameters (mm)</td>
<td></td>
<td></td>
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<tr>
<td>Overall motion (RMSD)</td>
<td>0.05 (0.02)</td>
<td>0.052 (0.02)</td>
<td>.68</td>
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<td>dL</td>
<td>0.009 (0.00)</td>
<td>0.01 (0.00)</td>
<td>.74</td>
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<tr>
<td>dP</td>
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<td>0.027 (0.01)</td>
<td>.84</td>
</tr>
<tr>
<td>dS</td>
<td>0.041 (0.02)</td>
<td>0.041 (0.02)</td>
<td>.95</td>
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<td>Pitch</td>
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<td>0.009 (0.00)</td>
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<tr>
<td>Yaw</td>
<td>0.012 (0.01)</td>
<td>0.012 (0.01)</td>
<td>.87</td>
</tr>
<tr>
<td>FD</td>
<td>0.054 (0.03)</td>
<td>0.049 (0.03)</td>
<td>.46</td>
</tr>
<tr>
<td>Time points remaining</td>
<td>287.9 (4.7)</td>
<td>285.9 (8.2)</td>
<td>.20</td>
</tr>
<tr>
<td>after motion scrubbings</td>
<td></td>
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<td></td>
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<tr>
<td>with FD &gt; 0.25 mm (of 290 time points collected)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Duration of sleep before scan (min)</td>
<td>64.6 (19.7)</td>
<td>67.7 (18.3)</td>
<td>.50</td>
</tr>
</tbody>
</table>

Note: Standard scores for overall cognitive, verbal, and nonverbal ability have a mean value equal to 100 (SD 15). ADOS = Autism Diagnostic Observation Schedule; ASD = autism spectrum disorder; dL = displacement in the left direction; dP = displacement in the posterior direction; dS = displacement in the superior direction; FD = frame-wise displacement; N/A = not applicable (TD controls did not receive the ADOS); RMSD = root-mean-square displacement; SD = standard deviation; TD = typically developing controls.
FIGURE 1  Functional connectivity maps with the amygdala. (A–C) Significant clusters of connectivity with left amygdala seed. (A) Within-group typical development (TD). (B) Within-group autism spectrum disorder (ASD). (C) Between-group difference (ASD < TD; blue circle marks the peak location of the cluster used in behavioral correlations). (D–F) Significant clusters of connectivity with right amygdala seed. (D) Within-group TD. (E) Within-group ASD. (F) Between-group difference (ASD < TD; blue circle marks the peak location of the cluster used in behavioral correlations). Note: All significant clusters are $p < .05$, corrected; clusters are overlaid on a representative 3.5-year-old structural brain image and are shown in radiologic convention (left = right).
and regions outside the occipital lobe, including the striatum, primary motor, and primary somatosensory cortices (Figure 2A). Direct between-group comparisons confirmed that the ASD group showed significantly weaker connectivity between V1 and sensorimotor regions, including pre- and post-central gyrus, striatum, and thalamus (Figure 2C).

Behavioral Correlations With Functional Connectivity
Correlations were generated to test whether connectivity between the amygdala and clusters showing significant group differences was associated with autism severity, as measured by the ADOS severity score of each participant with ASD. For the clusters with the greatest between-group difference (i.e., sensorimotor regions; see the blue circle marked in Figure 2C and Table S3 for additional anatomic details for the cluster; Table S3) was associated with sensory functioning (as measured by the Short Sensory Profile in the ASD and TD groups), while co-varying for overall cognitive ability and differences between groups. Across the 2 groups, weaker connectivity between V1 and sensorimotor regions was significantly correlated with greater hypersensitivity (lower scores) in the visual/auditory domain (r = 0.39; p = .04; co-varying for group and cognitive ability). This analysis was also conducted within the ASD group (in parallel to the approach with the ADOS analysis above) and the association held for the ASD group alone (r = 0.59; p = .02; co-varying for cognitive ability).

Conversely, V1 connectivity with sensorimotor regions was not related to autism severity in the ASD group (r = -0.07; p = .65; co-varying for overall cognitive ability), and amygdala connectivity with frontal lobe (r = 0.01; p = .72) and temporal lobe (r = 0.03; p = .75) clusters was not related to visual/auditory hypersensitivity across the
2 groups (co-varying for group and cognitive ability) or within the ASD group alone.

Post Hoc Analyses of Amygdala Volume
Given the evidence for volumetric differences in the amygdala in young children with ASD, the study tested whether amygdala volume was related to the observed differences in functional connectivity. Amygdala volumes were derived from the T1-weighted manual tracings. In the present sample, there were marginally significant group differences in amygdala volume only in the right hemisphere (ASD group: mean 1.58 cm³, standard deviation, 0.17; TD group: mean 1.49 cm³, standard deviation 0.17; p = .09), but not in the left hemisphere (ASD group: mean 1.45 cm³, standard deviation; TD group: mean 1.37 cm³, standard deviation 0.14; p = .38), after controlling for overall brain volume and age. Amygdala volume was not correlated with amygdala connectivity in any clusters from the between-group results (all Pearson correlations p > .20) in either diagnostic group.

DISCUSSION
In this study, preschool-age children with ASD had altered functional connectivity of the amygdala compared with age-matched TD children, particularly between the amygdala and areas important for social communication and repetitive behaviors, including the prefrontal cortex, temporal lobes, and striatum. While this study focused on the amygdala because of its known pathology in young children with ASD, we also sought to determine whether connectivity differences were specific to the amygdala. Therefore, a comparison functional connectivity analysis using V1 as the seed ROI was performed to determine whether connectivity differences were specific to certain neural systems. Similar to the amygdala findings, the ASD group showed overall weaker connectivity of V1, but the regional patterns of decreased connectivity were entirely different, occurring exclusively in sensorimotor regions, rather than the frontal and temporal lobes. While these findings add to the growing literature showing altered functional connectivity of the visual cortex in ASD, it is interesting to note that the neural circuitry involved in altered V1 connectivity was completely different than what was observed in the amygdala. This suggests that distinct behavioral associations could arise from the different patterns of altered connectivity.

Indeed, in addition to contrasting patterns of abnormal functional connectivity in ASD for the amygdala and V1, connectivity differences for these 2 regions were accompanied by specific and distinct behavioral associations. Although weaker connectivity between the amygdala and frontal lobe was associated with increased severity of the core diagnostic features of ASD, abnormal connectivity between visual cortex and sensorimotor regions was associated with sensory hypersensitivity. These findings highlight the importance of evaluating connectivity abnormalities in multiple neural systems to dissociate their relations with different functional domains of behavior in early childhood. This might be of particular importance in ASD, in which the interpretation of functional connectivity differences seen in the literature might not simply be stronger or weaker connectivity across the brain, but rather disrupted connectivity in specific functional networks.

To our knowledge, this is the first study to evaluate the functional connectivity of the amygdala in preschool-age children of any population (ASD or TD). Compared with typically developing controls, children with ASD had significantly weaker connectivity with several areas, including the mPFC and striatum. Several rs-fcMRI studies have reported aberrant striatal connectivity in school-age children with ASD, including specifically between the striatum and amygdala, and fMRI studies have shown abnormal function of the striatum. Abnormal connectivity and function of the striatum have been linked to altered social reward processing in ASD (for review, see Richter et al.). The mPFC regulates emotional responses triggered by the amygdala by providing contextual and experiential input to the amygdala, which in turn uses this information to interpret social stimuli and prepare behavioral and emotional responses. Abnormal function of the amygdala and mPFC in older children with ASD has been related to an exaggerated response of the amygdala to faces and has been linked to alterations in social reward and social motivation. In the present study of children at a young age close in time to diagnosis, weaker connectivity between the amygdala and mPFC was associated with increased autism severity. Thus, disrupted connectivity between the amygdala and mPFC during early development might reflect poorly coordinated co-activation between regions that underlie core features of ASD, particularly social-communicative impairments that might worsen during development.

One prominent framework of developmental changes in connectivity in ASD suggests that “young” individuals with ASD have over-connectivity that transitions to under-connectivity later in development. Although the present findings of under-connectivity in preschool-age children might seem inconsistent with this theory, it is worth noting that the existing framework is based on data in which school-age children (7–12 years of age) are considered “young” but do not extend to preschool-age children. The present findings are consistent with the only other rs-fcMRI study of preschool-age children with ASD, which reported under-connectivity at this age. Studies using diffusion tensor imaging also have indicated that white matter connectivity in toddlers does not resemble that of school-age children, which raises the importance of studying development from the youngest ages at a time near the age of diagnosis and following individuals longitudinally across the lifespan (for review, see Wolff and Piven). Given the dearth of fcMRI studies in preschool-age children, the present findings are worth consideration in the context of the developmental trajectories in typical development. Interestingly, the TD control group exhibited amygdala functional connectivity patterns similar to those seen in healthy adults, with robust connectivity between the amygdala and the mPFC, striatum, bilateral medial temporal lobes, and posterior ventral temporal lobes.
Resting-state fcMRI studies in TD individuals suggest that amygdala connectivity changes with age. Recent evidence suggests that school-age children have weaker amygdala connectivity with various regions, including the mPFC, compared with adults.\(^{59,60}\) While this growing literature has focused on school-age children through adulthood in typical development, previous studies have not included an evaluation of younger, preschool-age children, and the present findings indicate that amygdala-mPFC connectivity is reliably detected by 3 years of age in TD children. It is important to consider potential differences between resting-state functional connectivity during sleep, as data were acquired in the present study, versus while awake, as data have been acquired in other studies of older children and adults. Moreover, the developmental trajectory of functional connectivity might not follow a linear pattern from early to middle childhood and into adulthood. Additional longitudinal studies are needed to understand fully the developmental time course of amygdala connectivity.

The present study focused on a period of development (~3.5 years) that coincides with the average age of ASD diagnosis, before behavioral adaptation and treatment effects might influence the connectivity patterns of the amygdala. The present findings that preschool-age children with ASD have significantly weaker amygdala connectivity might represent a shift in the developmental time course in ASD that leads to altered connectivity compared with TD controls. Aberrant connectivity in ASD might be related to the longitudinal development of amygdala volume and connectivity. Evidence from cross-sectional structural MRI studies have shown that the amygdala undergoes an abnormal course of development in ASD that includes a period of early enlargement in the preschool years\(^{11-14}\) followed by a slower growth trajectory in preadolescence.\(^{13}\) Although direct correlations between amygdala volume and connectivity were not observed in the present study, future studies are needed to examine whether different subgroups of amygdala growth\(^{12}\) might be related to the longitudinal development of amygdala functional connectivity.

The findings presented in this study further the understanding of the role of the amygdala in the neuropathology of autism. Volumetric abnormalities of the amygdala have consistently been reported in young children with autism,\(^{11-14}\) and the present findings indicate that amygdala functional connectivity also is disrupted. Preschool-age children with ASD had weaker functional connectivity between the amygdala and regions important for social communication and language. Furthermore, connectivity related to the severity of core autism symptoms can be dissociated from connectivity related to sensorial difficulties. Although additional studies are clearly needed, these findings raise the potential for future treatment studies aimed at normalizing amygdala function and connectivity and decreasing social communication deficits in young children with ASD.

**REFERENCES**


5. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci. 2007;8:700-711.


