

# Amygdala Subregional Structure and Intrinsic Functional Connectivity Predicts Individual Differences in Anxiety During Early Childhood

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**Background:** Early childhood anxiety has been linked to an increased risk for developing mood and anxiety disorders. Little, however, is known about its effect on the brain during a period in early childhood when anxiety-related traits begin to be reliably identifiable. Even less is known about the neurodevelopmental origins of individual differences in childhood anxiety.

**Methods:** We combined structural and functional magnetic resonance imaging with neuropsychological assessments of anxiety based on daily life experiences to investigate the effects of anxiety on the brain in 76 young children. We then used machine learning algorithms with balanced cross-validation to examine brain-based predictors of individual differences in childhood anxiety.

**Results:** Even in children as young as ages 7 to 9, high childhood anxiety is associated with enlarged amygdala volume and this enlargement is localized specifically to the basolateral amygdala. High childhood anxiety is also associated with increased connectivity between the amygdala and distributed brain systems involved in attention, emotion perception, and regulation, and these effects are most prominent in basolateral amygdala. Critically, machine learning algorithms revealed that levels of childhood anxiety could be reliably predicted by amygdala morphometry and intrinsic functional connectivity, with the left basolateral amygdala emerging as the strongest predictor.

**Conclusions:** Individual differences in anxiety can be reliably detected with high predictive value in amygdala-centric emotion circuits at a surprisingly young age. Our study provides important new insights into the neurodevelopmental origins of anxiety and has significant implications for the development of predictive biomarkers to identify children at risk for anxiety disorders.

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**Key Words:** Amygdala, anxiety, children, fMRI, functional connectivity, machine learning

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Anxiety is a common emotional reaction and it normally serves as an adaptive mechanism for coping with challenging and stressful situations. High levels of sustained anxiety, however, can lead to increased vulnerability to mood- and anxiety-related disorders, especially during early childhood—a period when anxiety/depression symptoms begin to emerge (1–3). Individuals who experience anxiety more intensely during childhood are at increased risk for developing anxiety disorders later in life (3–5). Research in animals has shown that early anxiety has a significant impact on brain structure and function because of greater neuroplasticity and pruning during this period (3,6,7). However, little is known about the effects of anxiety on the brain during early childhood in humans, and the neurodevelopmental basis of individual differences in childhood anxiety remains poorly understood.

Research in animals has shown that stressful and anxious experiences during early childhood can induce long-lasting changes

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in brain structure and function. The most robust and consistent effects are observed in the amygdala (3,7), a region that is the core of the brain's emotion circuitry (8–10). Early life stress and anxiety have been linked to enlarged amygdala, particularly the basolateral nuclei, resulting from interplay of prolonged overactivity of stress-sensitive hormones and experience-dependent plasticity in the developing animal brain (7,11–13). In humans, amygdala enlargement has been reported in adults with generalized anxiety disorder (14–16), as well as healthy adults with high trait anxiety (17,18). However, findings in pediatric anxiety have been mixed (19–22). Critically, little is known about the relationship between anxiety and amygdala structure during early childhood, when the brain undergoes rapid changes and is likely to be highly vulnerable to the effects of stress and anxiety (5,7).

The amygdala encompasses multiple anatomical subregions with distinct roles in the modulation of cognitive and affective functions (8–10). The basolateral amygdala (BLA) and centromedial amygdala (CMA), the two most widely characterized subdivisions of the amygdala, subserve distinct functions via their unique connectivity profiles with cortical and subcortical regions (8). Specifically, the BLA plays an important role in perception and regulation of emotionally significant events via interactions with multiple brain systems, including sensory and perceptual association cortices, limbic-paralimbic affective systems, fronto-parietal attentional network, and medial prefrontal emotion regulation system (8–10,23). The CMA, in contrast, is essential for controlling automatic expressions of emotion, such as fear and freezing, through projections to brainstem, cerebellum, and sensorimotor system (8,24,25). In humans, similar dissociations between BLA and CMA functional circuits have been delineated using intrinsic functional connectivity analysis of the amygdala nuclei (14,25). Whether these individual nuclei and their large-scale intrinsic

functional connectivity are altered in childhood anxiety remains unknown.

Here, we investigate the effects of childhood anxiety on brain structure and connectivity in young children, within a restricted age range of 7 to 9 years, a period when anxiety-related traits and symptoms are first reliably identifiable (2). We used structural and resting-state functional magnetic resonance imaging (fMRI) techniques in conjunction with standardized neuropsychological assessments of childhood anxiety in 76 typically developing children. The Child Behavior Checklist (CBCL), a standardized parent-rated questionnaire with strong reliability and validity (26), was used to assess children's anxiety based on their general life experiences. An optimized voxel-based morphometry (VBM) approach was used to examine anxiety-related anatomical changes across the whole brain. Cytoarchitectonic mapping of amygdala nuclei was implemented to determine the specificity of alterations in BLA and CMA nuclei. Building on our previous developmental study in children (27), intrinsic functional connectivity analysis of resting-state fMRI data was used to examine the effects of childhood anxiety on large-scale functional connectivity of the amygdala and its two major subdivisions. Intrinsic functional connectivity analysis has emerged as a powerful systems neuroscience approach for delineating large-scale functional circuits and has been particularly useful for examining altered brain circuits associated with anxiety, stress, and mood in adults (28–30). Critically, we used a novel machine learning approach (31,32) to examine brain-based predictors of individual differences in childhood anxiety. Based on evidence from animal models and studies in human adults, we hypothesized that high childhood anxiety would be predicted by enlarged amygdala volume with the most prominent effects in the BLA. We further hypothesized that high childhood anxiety would be predicted by increased intrinsic functional connectivity of the amygdala, and the BLA in particular, with distributed brain regions involved in emotion processing.

## Methods and Materials

### Participants

A total of 76 children (38 boys, 38 girls) participated in this study after both parent and child gave written informed consent. Only children in the narrow age range of 7 to 9 years were included to minimize age-related variability. Children were typically developing, had no history of neurological/psychiatric disorders, and were not currently medication. Participant demographics are summarized in Table 1.

### Anxiety Assessment

Each child's anxiety level was obtained via parental reports using the standardized CBCL syndrome scales and the Diagnostic and Statistical Manual of Mental Disorders (DSM)-oriented anxiety problems scale (26) about 3 months before brain imaging data acquisition. The syndrome anxious/depressed scale measures multiple symptoms of anxiety and/or depression during childhood and has demonstrated strong reliability and validity (Table S1 in Supplement 1), allowing for an unbiased and dimensional assessment of varying levels of anxiety in children (26). Unless otherwise specified, the CBCL-based raw anxiety scores were used for brain imaging analyses. Scores from the DSM-oriented anxiety scale and other syndrome measurements (Table S2 in Supplement 1) were used in control analyses.

**Table 1.** Participant Demographics and Childhood Anxiety

	All Children	Boys	Girls
<i>N</i>	76	38	38
Age	8.2 ± .6	8.3 ± .6	8.2 ± .6
IQ	110.8 ± 11.0	110.2 ± 10.5	111.3 ± 11.7
Anxiety (Raw Score)			
Range	0–12	0–12	0–12
Mean	2.6 ± 2.7	2.3 ± 2.8	3.1 ± 2.9
Anxiety (T Score)			
Range	50–72	50–72	50–72
Mean	53.2 ± 5.2	53.0 ± 5.5	54.1 ± 5.9

Means (± standard deviation) of age, IQ, and childhood anxiety are listed for all children and for boys and girls separately. Raw score was computed by summing parental responses across items within the CBCL anxiety scale, and T score was determined for the appropriate age and gender as part of the internalizing syndrome factor.

CBCL, Child Behavior Checklist.

### Data Acquisition

Whole-brain high-resolution structural images were collected at a General Electric 3T Signa scanner (Milwaukee, Wisconsin), using a three-dimensional T1-weighted spoiled gradient-recalled inversion recovery magnetic resonance sequence. For resting-state fMRI data, children were instructed to keep their eyes closed and remain still for the duration of the 8-minute scan. Whole-brain functional images were acquired using a custom-built head coil with a T2\*-sensitive gradient echo spiral-in/spiral-out pulse sequence (based on blood oxygen level-dependent contrast) designed to increase signal-to-noise ratio and reduce signal dropout (33). Other details are provided in Supplement 1.

### Optimized VBM Analysis

Before preprocessing, structural images were checked for artifacts. Qualified images were then manually aligned to the conventional anterior commissure-posterior commissure space and the midsagittal plane. Voxel-wise cerebral volume across whole-brain structures was assessed using an optimized VBM method (VBM8; University of Jena, Germany, <http://dbm.neuro.uni-jena.de/vbm>). Images were resliced, spatially normalized to stereotaxic space, and then segmented into gray matter, white matter, and cerebrospinal fluid. Voxel-wise values of gray and white matter images were modulated by the Jacobian determinants derived from spatial normalization and smoothed with a 5-mm isotropic Gaussian kernel. Details are provided in Supplement 1.

Smoothed gray matter images were submitted to a second-level multiple regression analysis with childhood anxiety as a covariate of interest, while controlling for gender and age, to examine the relation between childhood anxiety and regional morphometry. The results were thresholded at a height threshold of  $p < .001$  and an extent threshold of  $p < .05$  with family-wise error correction using a nonstationary suprathreshold cluster-size approach based on Monte-Carlo simulations (34). Voxel-wise gray matter volume in observer-independent cytoarchitectonically defined amygdala subregions (see below) were extracted and submitted to linear regression and prediction analyses.

**Prediction Analysis.** A machine learning approach with balanced fourfold cross-validation combined with linear regression (35) was conducted to examine brain-based predictors of individual differences in childhood anxiety. Nonparametric testing was used to assess the performance of the regression model in predicting childhood anxiety and variations in regional morphometry. Childhood anxiety as a dependent variable and gray

matter volume in the amygdala subregions as an independent variable were treated as input to a linear regression algorithm. The  $r_{(\text{predicted, observed})}$ , a measure of how well the independent variable predicts the dependent variable, was first estimated by using a balanced fourfold cross-validation procedure. Data were divided into four folds so that the distributions of dependent and independent variables were balanced across folds (35). A linear regression model was built using three folds leaving out one fold, and this model was then used to predict the data in the left out fold. This procedure was repeated four times to compute a final  $r_{(\text{predicted, observed})}$  representing the correlation between the data predicted by the regression model and the observed data. Finally, the statistical significance of the model was assessed using a nonparametric testing approach. The empirical null distribution of  $r_{(\text{predicted, observed})}$  was estimated by generating 1000 surrogate datasets under the null hypothesis that there was no association between childhood anxiety and amygdala morphometry and connectivity. Other details are provided in Supplement 1.

**Region of Interest Definition of Amygdala and Its Subregions.** Amygdala region of interest (ROI) masks were created using cytoarchitecturally defined probabilistic maps of the entire amygdala and its two major nuclei, the BLA and CMA. Maximum probability maps were used to create these anatomical masks using the Anatomy toolbox (Institute of Neuroscience and Medicine, Jülich, Germany) (36). Voxels were included in the maximum probability maps only if the probability of their assignment to either one of two subdivisions was higher than any other nearby structures with greater than 40% likelihood. Given our a priori hypotheses of the BLA and CMA, these two nuclei were our primary focus. For completeness, we also conducted additional analyses of the superficial amygdala (SFA) using these same procedures.

### Intrinsic Functional Connectivity Analysis

Functional images were preprocessed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>; University College London, London,

United Kingdom). The first eight volumes were discarded for stabilization of magnetic resonance signal. Remaining images were realigned to correct for head motion, corrected for slice acquisition timing, spatially normalized to the Montreal Neurological Institute space, resampled into 2-mm isotropic voxels, and smoothed by convolving a 6-mm isotropic three-dimensional Gaussian kernel.

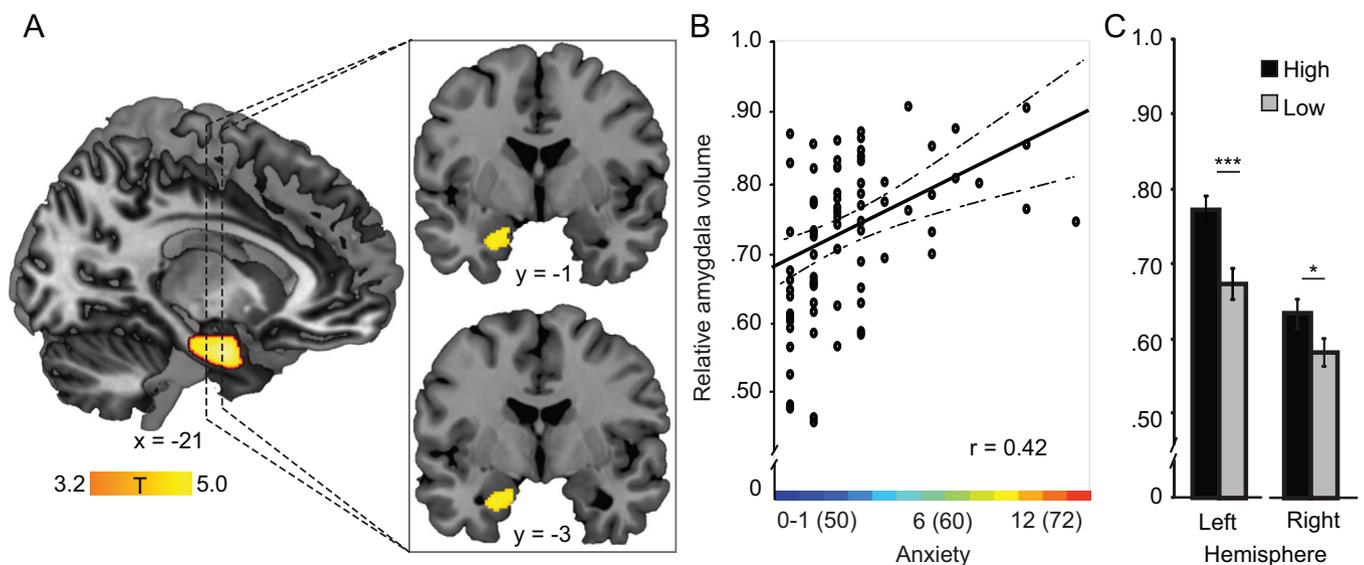
A seed-based correlational analysis was used to identify intrinsic functional connectivity of each seed with regions across the whole brain (37). Regional time series within each seed were extracted from bandpass-filtered images with a temporal filter (.008–.10 Hz) and submitted into the individual level fixed-effects analyses. A global signal regressor and six motion parameters were included as nuisance covariates to account for physiological and movement-related artifacts. Additional control analyses were conducted using the scrubbing procedure described by Power *et al.* (38) to correct effects related to micromotion (Supplement 1).

Connectivity maps of each seed were then submitted to a second-level multiple regression analysis, with childhood anxiety as the covariate of interest, while controlling for gender and age. Only clusters significant at a height threshold of  $p < .001$  and an extent threshold of  $p < .05$  with family-wise error correction are reported. To illustrate the relation between childhood anxiety and connectivity in specific target regions,  $\beta$  weights representing connectivity strength were extracted from significant clusters. Connectivity measures of the amygdala seeds with their respective targets were included in the prediction analysis to examine the predictive ability of individual differences in childhood anxiety.

## Results

### Participant Demographics and Anxiety Measures

Participant characteristics and anxiety levels determined using the CBCL (26) are summarized in Table 1. Children's anxiety levels



**Figure 1.** Relation between childhood anxiety and amygdala morphometry. **(A)** Three-dimensional sagittal view and two representative coronal slices of the left amygdala, which showed a significant positive correlation between childhood anxiety and voxel-wise gray matter volume. **(B)** Scatter plot depicting the correlation between childhood anxiety and gray matter volume of the left amygdala. Prediction analyses with machine learning algorithms revealed that amygdala volume can reliably predict childhood anxiety. Color bars on the x axis represent anxiety levels (normative  $T$  scores in parentheses) and the y axis represent gray matter volume of the anatomically defined amygdala. **(C)** Histogram showing gray matter volume of the left and right amygdala in children with high compared with low levels of anxiety. Error bars represent standard error of mean. \*\*\* $p < .001$ ; \* $p < .05$ .

varied considerably and the number of children with each anxiety level decreased monotonically (Figure S1 in Supplement 1), which is consistent with the distribution profile from the US National Survey (26). Boys and girls were equal in number and did not differ in age, IQ, or anxiety ( $p > .50$ ).

### High Childhood Anxiety Is Predicted by Enlarged Amygdala Volume

We first examined how childhood anxiety is associated with gray matter morphometry at the whole-brain level. A multiple regression analysis, using VBM procedures with childhood anxiety as the covariate of interest while controlling for gender and age, revealed that high childhood anxiety was associated with increased volume in the left amygdala (Figure 1A, Figure S2 in Supplement 1). No other region showed positive or negative correlations. Anxiety was not correlated with overall volume of gray matter, white matter, or cerebrospinal fluid (Table S3 in Supplement 1). Control analyses revealed no reliable correlations of amygdala volume with withdrawal/depressed or with other affective measures (Table S4 in Supplement 1). These results demonstrate a specific association between childhood anxiety and enlarged amygdala in children.

We then performed ROI analyses for the entire left and right amygdala to characterize the relationship between amygdala volume and childhood anxiety. We found a significant positive correlation of childhood anxiety with gray matter volume of the left ( $r = .42, p < .001$ ) and right ( $r = .23, p = .03$ ) amygdala (Figure 1B). To better illustrate amygdala enlargement as a function of anxiety levels, we used a median split to divide children into high versus low anxious groups. As shown in Figure 1C, this analysis revealed that high anxious children had larger left and right ( $t_{74} > 3.4, p < .001$ ) amygdala volume. Similar results were observed when using the DSM-oriented anxiety problems scale to compare high versus low anxious children (Figure S3 in Supplement 1).

We then conducted an additional analysis using machine learning algorithms with a balanced fourfold cross-validation procedure to assess how well amygdala volume predicts individual differences in childhood anxiety. This analysis revealed that the left amygdala volume predicted childhood anxiety

( $r_{(\text{predicted}, \text{observed})} = .33, p = .005$ ), but the right amygdala did not ( $r_{(\text{predicted}, \text{observed})} = .10, p = .17$ ) (Table S5 in Supplement 1). These results indicate that higher levels of childhood anxiety are predicted by larger left amygdala volume.

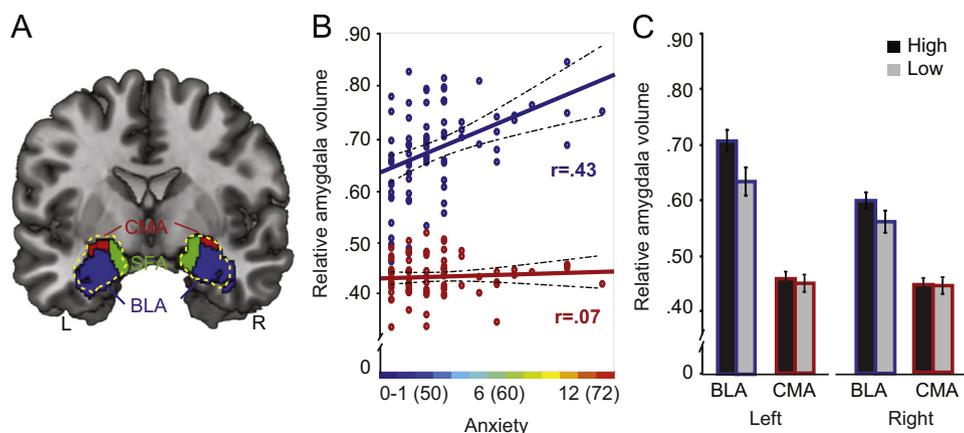
### High Childhood Anxiety Is Predicted by Enlargement of Specific Amygdala Nuclei

Next, we examined the relation between childhood anxiety and volume of specific amygdala nuclei. This analysis revealed the most pronounced enlargement in the left BLA ( $r = .43, p < .001$ ) but not in the left and right CMA ( $r < .1, p > .30$ ) (Figure 2A,B). A similar but less pronounced effect was observed in the left SFA ( $r = .33, p < .01$ ) (Figure S5 in Supplement 1). Additional analysis using a median split on anxiety scores yielded a similar pattern of results, with the largest effect in the left BLA ( $t_{74} = 3.50, p < .001$ ), an intermediate effect in the left SFA ( $t_{74} = 3.00, p < .01$ ), but null effects in the left and right CMA ( $t_{74} < 1.1, p > .25$ ) (Figure 2C).

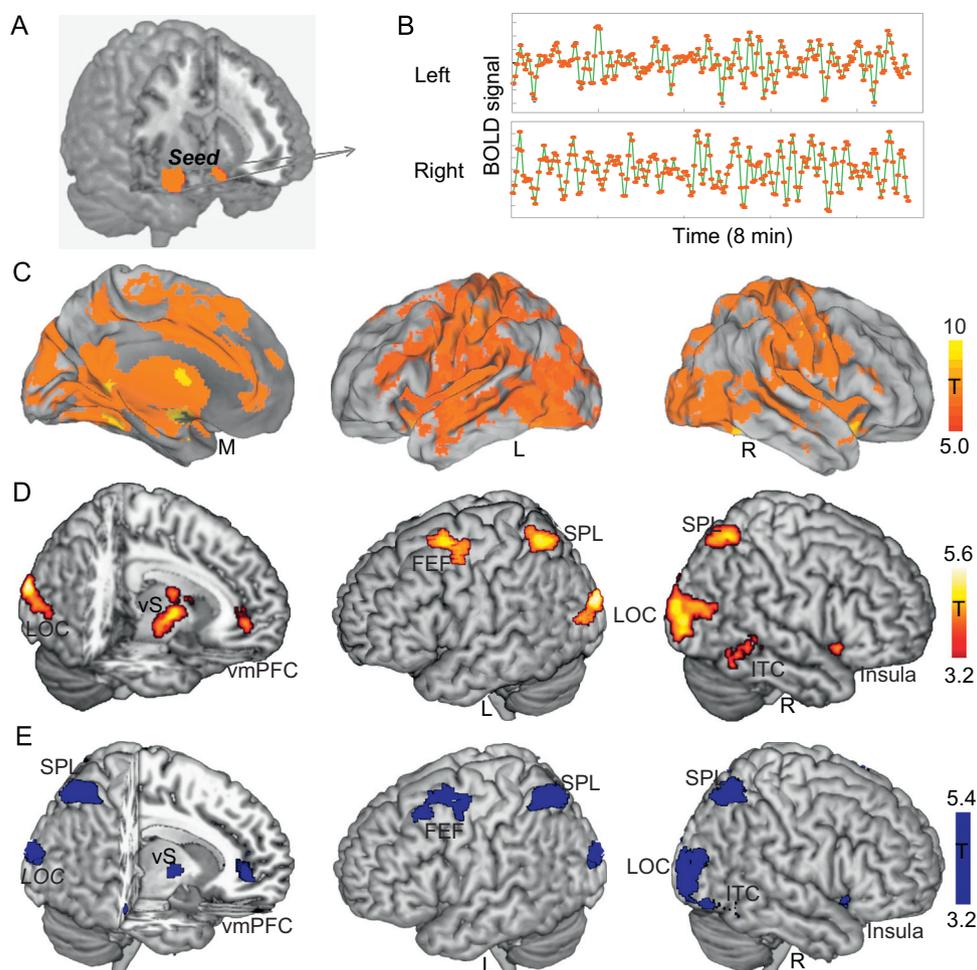
We then used a machine learning approach to examine whether BLA and CMA gray matter volume could predict childhood anxiety. These analyses revealed that volume of the left ( $r_{(\text{predicted}, \text{observed})} = .40, p = .001$ ) and right BLA ( $r_{(\text{predicted}, \text{observed})} = .22, p = .014$ ) predicted childhood anxiety (Table S5 in Supplement 1). Neither left ( $r_{(\text{predicted}, \text{observed})} = .03, p > .50$ ) nor right CMA ( $r_{(\text{predicted}, \text{observed})} = -.19, p = .16$ ) predicted childhood anxiety. The left SFA showed an intermediate pattern ( $r_{(\text{predicted}, \text{observed})} = .30, p < .01$ ), but the effect in right SFA was not significant ( $r_{(\text{predicted}, \text{observed})} = .19, p = .10$ ). Together, convergent results using regression and prediction analyses indicate that high childhood anxiety is associated with enlarged left BLA and SFA volume, with the left BLA as the strongest predictor.

### High Childhood Anxiety Is Predicted by Increased Large-Scale Amygdala Connectivity

We then examined how childhood anxiety alters intrinsic functional connectivity of the amygdala (Figure 3A–C; Figure S5 and Tables S6 and S7 in Supplement 1). This analysis revealed that higher childhood anxiety is associated with greater functional connectivity, i.e., hyperconnectivity, of the left amygdala



**Figure 2.** Relation between childhood anxiety and amygdala subregional morphometry. **(A)** Representative coronal slices of the three major subdivisions of amygdala nuclei—basolateral (BLA, blue), centromedial (CMA, red), and superficial (SFA, green) amygdala. A yellow line outlines the whole amygdala. Data regarding SFA is shown in Figure S3 in Supplement 1. **(B)** Scatter plots depicting the correlations of childhood anxiety with gray matter volume of left BLA and CMA. Prediction analyses further revealed that left BLA volume is the strongest predictor of childhood anxiety. **(C)** Histogram showing gray matter volume of the two major amygdala subdivisions in children with high compared with low levels of anxiety. Error bars represent standard error of the mean. \*\*\* $p < .001$ . L, left; R, right.



**Figure 3.** Relation between amygdala intrinsic functional connectivity and childhood anxiety. **(A)** Anatomically defined overall amygdala seeds in the left and right hemispheres. **(B)** Time series of spontaneous functional magnetic resonance imaging signal fluctuations in each seed over 8 minutes from one participant. **(C)** Widespread brain areas that showed significant intrinsic functional connectivity with the amygdala derived from a seed-based functional connectivity analysis of resting-state functional magnetic resonance imaging data. **(D)** Brain regions that showed significantly greater functional connectivity of the left amygdala in children with higher levels of anxiety. Significant positive correlations were observed in regions important for 1) sensory and perceptual processing (i.e., lateral occipital cortex [LOC]; inferior temporal cortex [ITC]); 2) attentional control (i.e., frontal eye field [FEF]; superior parietal lobe [SPL]); 3) motivation and reward (i.e., ventral striatum [vS]); and 4) saliency detection and emotion regulation (i.e., anterior insula; ventromedial prefrontal cortex [vmPFC]). **(E)** Brain regions that showed significantly greater functional connectivity of the left basolateral amygdala (in blue) in children with higher levels of anxiety. BOLD, blood oxygen level-dependent; L, left; M, medial; R, right.

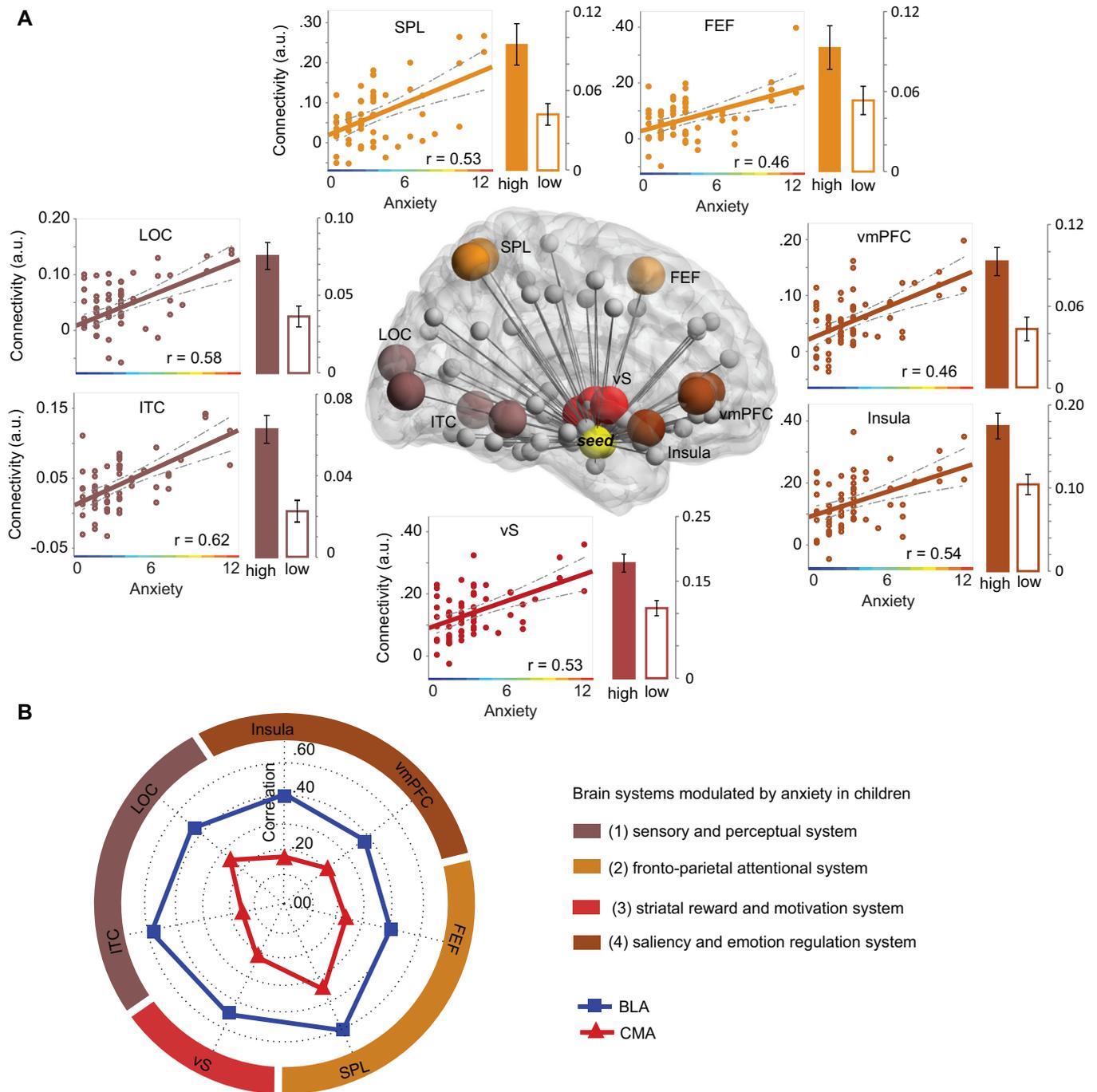
with multiple brain areas (Figure 3D). These areas included 1) lateral occipital and inferior temporal cortices in sensory association cortex; 2) frontal eye field and superior parietal lobe, which are part of the dorsal fronto-parietal attentional network; 3) putamen and ventral striatum in the basal ganglia, along with thalamus, hypothalamus, and midbrain; and 4) anterior insula and ventromedial prefrontal cortex (vmPFC) in paralimbic areas (Figure S5A–D, Table S6 in Supplement 1). Critically, our prediction analyses showed that intrinsic functional connectivity of the left amygdala with each of these regions can reliably predict individual differences in childhood anxiety (Figure 4A; Table S8 in Supplement 1).

We also conducted a parallel analysis with the right amygdala as the seed ROI. In this case, higher levels of childhood anxiety were correlated with greater connectivity with the right anterior insula and basal ganglia (Figure S7, Table S9 in Supplement 1). To address concerns about the effects of micromotion on brain functional connectivity, we conducted additional analyses using

the procedures described by Power *et al.* (38). This analysis yielded a weaker, yet almost identical, pattern of results (Figure S6A'–D', Table S10 in Supplement 1). These results indicate that high childhood anxiety is associated with hyperconnectivity of the amygdala with multiple distributed areas.

#### High Childhood Anxiety Is Predicted by Increased Basolateral Amygdala Connectivity

We further examined intrinsic functional connectivity of the specific amygdala nuclei (Figures S8 and S9 in Supplement 1) in relation to childhood anxiety. This analysis revealed that higher levels of childhood anxiety were associated with greater connectivity of the left BLA subdivision with multiple brain areas in sensory-perceptual association cortex, dorsal fronto-parietal network, ventral striatum, insula, and vmPFC (Figure 3E). In contrast, CMA-based connectivity was not associated with childhood anxiety. Neither BLA nor CMA showed reliable negative correlations with childhood anxiety. These results indicate that high childhood



**Figure 4.** Schematic illustration of relation between childhood anxiety and functional connectivity of the amygdala and its subregions. **(A)** Brain regions (represented by color-coded nodes) that showed a significant correlation between childhood anxiety and functional connectivity with the left amygdala. Target regions include 1) lateral occipital cortex (LOC) and inferior temporal cortex (ITC) in sensory association cortex; 2) frontal eye field (FEF) and superior parietal lobe (SPL) that are part of dorsal fronto-parietal network; 3) ventral striatum (vS) in basal ganglia; and 4) insula and ventromedial prefrontal cortex (vmPFC) in paralimbic system. Gray nodes and lines represent the functional connectivity network of the amygdala seed (shown in yellow), with peaks of significant clusters from the intrinsic functional connectivity analysis. Scatter plots depict positive correlations between anxiety levels (x axes) and connectivity strength between the left amygdala and each target region (y axes). Bar graphs depict connectivity strength between the left amygdala and each target region in high versus low anxious groups using a median-split. The bar graphs are shown for visualization. **(B)** Polar plot illustrating the relation between childhood anxiety and amygdala subregional connectivity with multiple regions broadly classified into four functional brain systems: 1) sensory and perceptual processing (dark brown); 2) attentional control (orange); 3) motivation and reward (red); and 4) saliency detection and emotion regulation (light brown). Prediction analyses revealed that left basolateral amygdala (BLA) functional connectivity was the strongest predictor of childhood anxiety. CMA, centromedial amygdala.

anxiety is specifically associated with hyperconnectivity of the left BLA but not CMA (Figure 4B). Additional analysis revealed that SFA connectivity showed a similar pattern of results as the BLA,

except that no significant effects of anxiety were observed in the left dorsal fronto-parietal network with SFA (Figure S10 in Supplement 1).

To examine the predictive ability of amygdala subregional connectivity to childhood anxiety, we conducted machine learning-based prediction analyses using BLA-based and CMA-based connectivity with target regions of interest (Figure 3D). These analyses revealed that functional connectivity of the left BLA with sensory-perceptual association cortex, dorsal frontoparietal network, ventral striatum, insula, and vmPFC most reliably predicted individual differences in childhood anxiety (Table S11 in Supplement 1). Taken together, these results converge on our anatomical findings and further indicate that the left BLA connectivity is a reliable predictor of childhood anxiety.

## Discussion

To our knowledge, this is the first study to demonstrate that even in a subclinical sample of children as young as 7 to 9 years of age, childhood anxiety is associated with and can be reliably predicted by the morphometry and intrinsic functional connectivity of specific amygdala nuclei. Specifically, children with high levels of anxiety showed enlarged amygdala and increased intrinsic connectivity with distributed regions, with the BLA emerging as the strongest predictor. Our findings provide novel evidence that BLA enlargement and its hyperconnectivity with multiple functional systems is a prominent feature of childhood anxiety. These effects were specific to anxiety, as withdrawn/depression and other measures (i.e., somatic complaints, social problems, thought problems, attention problems, rule breaking, and aggressive behaviors) did not show such a relationship with the amygdala structure or connectivity.

Amygdala enlargement observed here is strikingly similar to previous findings in 8- to 16-year-old children and adolescents with generalized anxiety disorder (20). Although two studies in 7- to 14-year-old individuals with posttraumatic stress disorder reported no differences in amygdala (19,22), amygdala enlargement has been consistently reported in adults with generalized anxiety disorder (15,16,39) and healthy adults with high trait anxiety (17,40). Together, our findings converge on and extend findings from clinical studies and provide new evidence that early childhood anxiety is associated with enlarged amygdala, even in children as young as ages 7 to 9. Critically, cytoarchitectonic mapping and machine learning approaches revealed the left BLA volume to be the strongest predictor of childhood anxiety. Unlike adults with generalized anxiety disorder (14), anxious children in our study did not demonstrate increased volume in the CMA. Although the definitive mechanisms underlying the differential BLA enlargement in children remain an open question, our findings suggest that heterogeneous abnormalities in specific amygdala nuclei may underlie the development of vulnerability to anxiety disorders.

The effects of anxiety on amygdala growth have only been demonstrated in animal models of early life stress (6,7,41). These studies have determined increased formation of new synapses in the BLA in rodents chronically exposed to stressful and anxious environments (12,42,43). In particular, stress and anxiety in early life are associated with greater dendritic arborization and aberrant pruning of synapses over development, leading into an increased rate of growth in amygdala (6). Such increases have been linked to prolonged activation of stress hormones, such as cortisol, which act directly on the BLA (7,41,43) and lead to increased anxiety-like behaviors (44). Extrapolating from these studies in animals, we hypothesize that similar neuronal and hormonal mechanisms might underlie the amygdala enlargement observed in our study. This view is supported by a recent study that reported elevated levels of cortisol and amygdala

enlargement in 10-year-old children who experienced high levels of stress and anxiety arising from early exposure to maternal depressive symptoms (41).

In conjunction with amygdala enlargement, we found that high childhood anxiety is predicted by increased connectivity of the amygdala, and the BLA in particular, with multiple distributed brain regions involved in emotion. These regions can be broadly classified into four functional systems: 1) sensory and perceptual system (lateral occipital and inferior temporal cortices); 2) frontoparietal attentional system (frontal eye field and superior parietal lobe); 3) striatal reward and motivational system (ventral striatum); and 4) saliency and emotion regulation system (anterior insula and vmPFC). Aberrations in connectivity of the amygdala with each of these brain systems have been reported in previous studies of adults with various anxiety disorders (14,39,45–49).

The increased connectivity with sensory and perceptual system and fronto-parietal attentional system is consistent with reports of a vigilance-attention bias in anxiety. Hypervigilance, characterized by hyperreactivity in sensory and perceptual association areas, for instance, has been reported by several studies in anxious adults (50–52) and patients with anxiety disorders (39,46). Sustained anxiety can also lead to information processing biases, with more rapid orientation of attention to negatively valenced stimuli (45,53). Alterations in these systems may underlie a vigilance-attention bias (45,54) in anxious children.

The third system involves the ventral striatum, which is important for incentive processing and reward-seeking behavior (23,55). Sustained anxiety in children may bias this system toward withdrawal or avoidance behavior to alleviate anxious states (56). The fourth system, involving the anterior insula and vmPFC, plays a critical role in saliency detection and regulation of emotion. Consistent with our findings in children, increased amygdala connectivity with the insula has been associated with high trait anxiety in adults (57,58). The vmPFC acts as a hub that links multiple cortical and subcortical systems to determine the affective value of environmental events and to generate the appropriate adaptive behaviors (23,59). Dysfunction of the vmPFC has been associated with aberrant emotion regulation in patients with anxiety disorders (39,46). Although both hyperactivation and hypoactivation in amygdala-vmPFC circuitry in emotion processing have been reported in anxiety disorders, our finding in children is consistent with amygdala-vmPFC hyperconnectivity during resting state in healthy adults with high anxiety (30). Altogether, our findings suggest that amygdala hyperconnectivity in anxious children has a neuroanatomical profile similar to those seen in adults with high anxiety (17,18) or anxiety disorders (39,46). Our data add to emerging neurocognitive models suggesting that vigilance-attention and emotion regulation systems are altered in anxious individuals (45,53,54). Importantly, our findings show for the first time that such alterations may manifest in children as young as 7 to 9 years of age.

Interaction between stress exposure and altered amygdala circuitry may exacerbate vulnerability toward anxiety disorders, as suggested by diathesis-stress models of mental health (60). Our findings suggest that a possible diathesis of anxiety may be alterations in the amygdala structure and connectivity leading to the emergence of trait-like susceptibility to anxiety. On the one hand, interactions between such a diathesis, stress exposure, and experience-dependent plasticity in the developing brain may lead to negative consequences such as anxiety-related disorders. On the other hand, these changes may confer some adaptive advantages for developing balanced strategies to cope with challenging and stressful situations in real life (61). Future

longitudinal studies are required to address the long-term effects of childhood anxiety on brain development, especially during highly vulnerable periods of childhood and adolescence (62,63). Our study represents an important first step in characterizing altered brain systems and identifying predictive biomarkers of individual differences in childhood anxiety at a young age.

In conclusion, our study shows that amygdala enlargement and functional hyperconnectivity are predictive of high levels of anxiety in children as young as 7 to 9 years of age. The most prominent enlargement and functional hyperconnectivity were observed in the left BLA, an amygdala nucleus whose large-scale functional circuits are important for emotion perception and regulation. Understanding the influence of childhood anxiety on amygdala circuits, as identified here, will not only help promote the development of biomarkers to identify at-risk children for early intervention, but also enhance our understanding of the neurodevelopmental origins of anxiety-related psychopathology.

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