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

Shaozheng Qin, Christina B. Young, Xujun Duan, Tianwen Chen, Kaustubh Supekar, and Vinod Menon

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.

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

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 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

From the Departments of Psychiatry and Behavioral Sciences (SQ, XD, TC, KS, VM) and Neurology and Neurological Sciences (VM) and Program in Neuroscience (VM), Stanford University School of Medicine, Stanford, California; and Department of Psychology (CBY), Northwestern University, Evanston, Illinois.

Address correspondence to Shaozheng Qin, Ph.D., Stanford University School of Medicine, Department of Psychiatry and Behavioral Sciences,1070 Arastradero Road, Suite #220, Palo Alto, CA 94304; E-mail: szqin@stanford.edu.

Received May 23, 2013; revised Sep 4, 2013; accepted Oct 3, 2013.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2013.10.006

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
N	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 stereotactic 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