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Altered Development of Amygdala-Anterior Cingulate Cortex Connectivity in Anxious Youth and Young Adults

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Abstract

Background—Development of cortico-amygdala circuitry underlies the maturation of emotion processing and regulation, and age-related changes in amygdala connectivity with anterior cingulate cortex (ACC) have been shown to mediate normative developmental decreases in anxiety. It remains unclear whether developmental changes in this circuitry relate to pathological anxiety in youth. The current functional magnetic resonance imaging (fMRI) study addresses this question by examining amygdala functional connectivity in anxious and healthy individuals spanning the developmental period from childhood through adulthood.

Methods—Youth and young adults (age 7–25) with current anxiety disorders ($n=57$) and healthy comparisons ($n=61$) completed an fMRI emotional face processing task known to elicit amygdala activation in youth and adults. We examined interaction effects of anxiety group and age on amygdala connectivity with frontolimbic regions during processing of happy, angry, and fearful faces.

Results—Anxiety interacted with age to predict amygdala-ACC connectivity across emotional faces. Among healthy comparisons, age was negatively related to connectivity. In contrast, age was positively associated with amygdala-ACC connectivity in the anxious group. Group effects

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were also observed on amygdala connectivity with midcingulate and middle frontal gyri. Effects of anxiety and age on amygdala activation were not significant.

Conclusions—Results indicate that anxiety is characterized by altered patterns of age-related changes in amygdala connectivity during emotional face processing. Positive associations between age and amygdala-ACC connectivity among anxious youth and young adults may indicate failure to establish early bottom-up connections in childhood and/or less top-down regulation of the amygdala into adulthood.

Keywords

anxiety; development; amygdala; emotional faces; functional connectivity; anterior cingulate cortex

Introduction

Anxiety disorders are among the most prevalent disorders in youth and are characterized by abnormalities in emotion processing (1; 2), raising the importance of identifying the neural pathology underlying these deficits (3). Emotion processing involves the amygdala, which signals the presence of threat and salient information, as well as the prefrontal cortex (PFC) and anterior cingulate cortex (ACC), which appraise stimuli and monitor and regulate emotion (4–6). There is evidence of a mismatch in the development of cortico-amygdala circuitry, with amygdala maturing earlier in childhood or adolescence, and PFC continuing to develop into adulthood (7; 8). That is, systems involved in identifying emotional information develop relatively early, with regions involved in top-down regulation of emotions maturing later.

Cortico-amygdala circuitry appears to be disrupted in anxiety disorders (6; 9–11), particularly when processing threatening information. For example, anxiety disorders in youth (including generalized anxiety disorder [GAD], social anxiety disorder, and panic disorder) have been associated with greater amygdala reactivity to threatening faces (12–16). In addition, anxious youth exhibit abnormalities in frontal regions during processing of emotional information, including enhanced activation in ventrolateral PFC (vlPFC) and ACC in response to threatening faces, possibly indicating the need for greater activation in these regions to regulate emotional responses (13; 14; 16).

Along with activation, amygdala interactions with PFC and ACC are particularly important for emotional reactivity and regulation (9). Recent work has identified age-related differences in amygdala-ACC connectivity that may underlie the development of emotion regulation abilities. In a sample of 4- to 22-year-old healthy participants, connectivity between amygdala and ACC during viewing of fearful faces decreased with age, such that young children showed *positive* connectivity while adolescents and adults showed *negative* connectivity, possibly indicating greater bottom-up signaling of the ACC by the amygdala in childhood and greater top-down regulation of the amygdala into adulthood (17). In addition, development of amygdala-ACC connectivity mediated associations between age and normative developmental declines in separation anxiety (17). Though there is evidence of abnormal amygdala connectivity in youth with clinical anxiety (14; 18), age-related

differences in amygdala-ACC connectivity have yet to be examined in anxious youth, an important direction for understanding the emergence of atypical brain development.

In contrast to the literature on amygdala connectivity with ACC, there is evidence of normative developmental increases in amygdala connectivity with other frontal regions. For example, a study of 10- to 24-year-olds found age-related increases in amygdala and hippocampus connectivity with orbitofrontal cortex and ventrolateral PFC during emotional image viewing (19). In addition, in a study of 8- to 18-year-olds, healthy youth exhibited age-related increases in amygdala connectivity with ventromedial PFC during threat processing, while youth with post-traumatic disorder (PTSD) exhibited age-related decreases in connectivity (20).

Given evidence of developmental changes in amygdala connectivity with frontal regions, we sought to compare age-related differences in youth and young adults (age 7 to 25 years) with clinical anxiety to healthy comparisons. Participants included 61 healthy comparisons and 57 youth and young adults with primary diagnoses of GAD, social anxiety disorder, or separation anxiety disorder, which tend to have earlier ages of onset than other anxiety disorders and often co-occur (21). Because faces are developmentally-appropriate and relevant to adaptive social behavior, we used a functional magnetic resonance imaging (fMRI) emotional face matching task known to elicit amygdala activation in both youth and adults (18; 22). We hypothesized that age would be *negatively* related to amygdala-ACC in healthy controls, consistent with prior work (17), but this developmental process would be disrupted in anxiety. We evaluated neural responses to angry, fearful and happy faces in order to evaluate whether effects are apparent for broad emotion processing or threat processing specifically. Additional analyses examined age and group effects on amygdala connectivity with other frontolimbic regions.

Methods and Materials

Participants

The initial sample included 136 participants, but 18 participants were excluded for movement (see Preprocessing), leaving a final sample of 57 anxious participants and 61 healthy comparisons. The sample was 57.6% Caucasian, 13.6% African American, 12.7% Latino, and 16.1% Asian or Pacific Islander. Procedures were approved by the Institutional Review Boards (IRB) at University of Illinois at Chicago (UIC) and University of Michigan (UM), and participants were recruited at both sites (Table 1). Adult participants and parents of children under the age of 18 provided written informed consent and verbal assent was obtained from minors. We previously reported on typical age-related changes in brain activation and amygdala connectivity during emotional face processing in this sample of healthy volunteers (23) and on neural processing of emotional faces in separate studies of anxious youth (18) and young adults (24; 25). In this paper, we extend this work by combining pediatric and adult samples to examine age-related changes in amygdala connectivity in anxiety.

Diagnostic Assessment

Participants were screened for current and lifetime psychopathology using a semi-structured interview administered by Master's-level or Doctorate-level clinicians: the Kiddie Schedule of Affective Disorders and Schizophrenia (26) for children and adolescents and the Structured Clinical Interview for DSM-IV (27) for adults. Anxious participants had current primary diagnoses of GAD, social anxiety disorder, or separation anxiety disorder. Participants with secondary comorbid anxiety or depressive disorders were eligible, but those with histories of bipolar disorder, schizophrenia, intellectual disability, or pervasive development disorders, as well as those with current substance use disorders, severe current depression, or current suicidal ideation were excluded. Participants were not taking psychotropic medications or participating in psychotherapy at the time of the study.

Anxiety Symptoms

Social anxiety severity—As the majority of the anxious group met criteria for current social anxiety disorder, children and adolescents completed the interviewer-administered Liebowitz Social Anxiety Scale for Children and Adolescents (LSAS-CA; 28), and adults completed the adult version of the LSAS (29). Separate z-scores were computed for each version in order to compare relative level of anxiety severity across both measures. LSAS data were missing for 2 healthy comparisons.

General anxiety severity—Children and adolescents completed the Pediatric Anxiety Rating Scale (30), an interviewer-rated measure of anxiety severity. Because a comparable measure of anxiety severity was unavailable for adult participants, only children and adolescents were included in analyses of general anxiety severity. PARS data were unavailable for 19 healthy comparisons and 28 anxious participants.

Emotional Face Processing Task

Participants performed a modified version of the emotional face processing task by Hariri et al. (22), previously used to assess amygdala reactivity in anxious youth and adults (18; 31). The task consisted of 18 blocks: 9 blocks of matching facial expressions, interspersed with 9 blocks of matching shapes. Each block lasted 20 s, containing 4 sequential matching trials (5 s each). For face matching, participants viewed three faces and were instructed to match the emotion of the target face on the top with one of two faces on the bottom. The target (top) and matching probe (bottom) displayed emotional expressions; the foil face (bottom) displayed a neutral expression on every trial. Three blocks of each expression (i.e., angry, fearful, and happy) were included. For shape matching, participants were instructed to match simple shapes (i.e., circles, rectangles, and triangles).

MRI data

Acquisition—MRI data were collected on 3 Tesla GE scanners with 8-channel head coils at two sites. At UM, functional data were collected with a gradient-echo reverse spiral acquisition with two sets of imaging parameters: repetition time (TR) 2 s, echo time (TE) 30 ms, flip angle 90°, field of view (FOV) 22 x 22 cm², acquisition matrix 64 x 64, 3-mm slice thickness with no gap, 43 axial slices; or TR 2 s, TE 30 ms, flip angle 77°, FOV 24 x 24

cm², acquisition matrix 64 x 64, 5-mm slice thickness with no gap, 30 axial slices. At UIC, functional data were acquired using gradient-echo echo-planar imaging (EPI) sequence with the following parameters: TR 2 s, TE minFull [~25 ms], flip angle 90°, FOV 22 x 22 cm², acquisition matrix 64 x 64, 3-mm slice thickness, 44 axial slices. Because slices differed among participants, we examined relationships with variables of interest and included number of slices as a covariate in post-hoc analyses. Number of slices was related to age, $r(116) = -.57$, $p < .001$, but not anxiety group or the interaction between group and age ($ps > .45$; see Supplementary Information for covariate analyses).

Preprocessing—Functional images were preprocessed in SPM8 (Wellcome Trust Centre for Neuroimaging, <http://www.fil.ion.ucl.ac.uk/spm/>) for slice timing correction, spatial realignment, image normalization, resampling at a 2 x 2 x 2 mm³ voxel size, and 8-mm Gaussian imaging smoothing. Volumes with motion artifacts exceeding 3 mm and/or with a z-threshold of 6 were removed using Artifact Detection Tools (https://www.nitrc.org/projects/artifact_detect/). Participants with more than 10 outlier volumes were excluded from the study ($n = 17$: 10 anxious and 7 healthy comparisons). Volumes with greater than 0.5 mm framewise displacement (FD) were censored (32) using FSL (33), and one healthy comparison was excluded for exceeding FD thresholds on >30% of scans, yielding the final sample of 118 participants. On average, 4.18 ($SD = 8.45$) volumes were censored for FD. Mean FD and number of volumes censored were related to age, $r(116) = -.50$, $p < .001$, and, $r(116) = -.43$, $p < .001$, respectively, but did not differ between groups ($ps > .58$), and the interactions between age and anxiety group were not significant ($ps > .20$).

Data Analysis

Brain activity—First-level within-subject analysis was performed with a general linear model with six regressors: faces (angry, fearful, and happy) and shapes (circle, rectangle, and triangle). Nuisance regressors for 6 motion parameters and outlier volumes were included to correct for motion artifacts. For each participant, contrast images of brain activity (e.g., faces vs. shapes) were generated for further second-level between-subject analysis.

Functional connectivity—First-level functional connectivity maps were generated for each individual using generalized Psychophysiological Interaction (gPPI) analysis (34) in SPM8 to examine voxelwise functional coupling between amygdala and other brain regions. Two seed regions, left and right amygdala, were created based on anatomically defined Automated Anatomical Labeling (AAL) atlas (35). Right and left amygdala were examined separately given evidence of distinct patterns of connectivity with some regions (36). The hemodynamic response function was deconvolved to derive the neural signal and to compute the interaction term (seed time series x task condition). First-level models included mean time series of the seed region, task conditions (angry, fearful, happy faces, and shapes), interaction variables (seed times series x task condition), and motion parameters and outlier volumes.

Second-level analyses—ANCOVAs were performed in SPM8 to investigate 2-way and 3-way interactions between group (anxious vs. healthy comparison), emotion condition

(angry, fearful, and happy), and age. Given evidence of sex differences in amygdala activation during emotional face processing (37) and to control for methodological variables, sex and study site/scanner were included as covariates in all second-level analyses.

Given our focus on amygdala connectivity, we first examined group and age effects on activation in an amygdala ROI (anatomically defined using AAL; 35). Supplementary analyses examined activation in frontolimbic regions. Next, group and age effects were examined on amygdala connectivity with frontolimbic regions in an anatomically-defined mask, created using WFU PickAtlas (38) encompassing frontal lobes, amygdala, cingulate, insula, hippocampus, and parahippocampus (426,800 mm³; Figure S1). To correct for multiple comparisons, joint height and extent thresholds were determined via Monte Carlo simulations (10,000 iterations) and applied to second-level results for corrected $p < .05$ (AlphaSim, AFNI; 39). Thresholds were 53 voxels for activation and 58 voxels for connectivity. To interpret interactions, parameter estimates (β weights) of significant clusters were extracted from individual connectivity maps using MarsBar (40).

Results

Participant Characteristics

Demographics and rates of psychopathology are presented in Table 1. Anxious and healthy comparison groups did not significantly differ on sex, race, site of data collection, or age ($ps > .29$). Participants recruited at UM compared to UIC did not significantly differ on distribution of anxious vs. healthy comparison or age ($ps > .19$).

Behavioral Results

Behavioral data were unavailable for 10 participants due to a technical error, and 3 participants were excluded from behavioral analyses for low accuracy (<60%)¹. Mixed-design ANCOVAs were computed with Condition (angry, fearful, happy faces, or shapes) as the within-subjects factor to examine main effects of Group, Age, and Group x Age interactions on accuracy and mean reaction time (RT), controlling for sex and site of data collection. No significant effects of Group or Group x Age interactions were observed ($ps > .16$). For accuracy, the Condition x Age interaction was significant, $F(3, 297) = 10.26$, $p < .001$, such that age was positively related to accuracy for angry faces, $r(101) = .36$, $p < .001$, but not significantly associated with accuracy for fearful faces, happy faces, or shapes ($ps > .07$). Increasing age was associated with faster RT across all conditions, $F(1, 99) = 87.07$, $p < .001$.

Brain Activity

Amygdala—Significant bilateral amygdala activation to emotional faces versus shapes was observed in the anxiety group (left amygdala $t = 9.68$, peak voxel: $-28, -4, -20$, volume: 1592 mm³; right amygdala $t = 9.47$, peak voxel: $22, -2, -18$, 1840 mm³) and healthy comparisons (left amygdala $t = 9.44$, peak voxel: $-20, -2, -20$, 1600 mm³; right amygdala $t = 9.71$, peak voxel: $24, -2, -22$, 1840 mm³). The main effects of Group and Age, as well as

¹Second-level amygdala functional connectivity analyses were computed excluding the 3 participants with low accuracy and no substantive changes in results were observed.

the 2- and 3-way interactions were not significant for clusters within the amygdala ROI (small volume corrected family-wise error $ps > .05$).

Although not the focus of the current analysis, we report significant interaction effects on activation across frontolimbic regions in Supplementary Information (Table S1).

Amygdala Functional Connectivity

Table 1 presents effects of Age and Group on amygdala connectivity. Group x Age interactions were observed for both right and left amygdala connectivity with ACC across emotional faces. A Group x Age x Emotion interaction was observed for left amygdala connectivity with midcingulate gyrus (MCG), and a Group x Emotion interaction was observed for left amygdala connectivity with middle frontal gyrus (MFG). Post-hoc analyses were computed on extracted clusters to interpret interactions and examine associations with primary diagnoses and symptom severity. Additional post-hoc analyses examined whether effects were driven by behavioral performance, motion artifacts, or study site/scanner, with no substantive changes in results observed when controlling for these potential confounds (see Supplemental Results).

Amygdala-ACC connectivity—Partial correlations were computed to examine relationships between age and amygdala-ACC connectivity to emotional faces within each group. For healthy comparisons, age was *negatively* related to both left amygdala, $r(57) = -.34$, $p < .01$, and right amygdala, $r(57) = -.29$, $p = .03$, connectivity with ACC, controlling for sex and site/scanner. In the anxiety group, age was *positively* related to left amygdala connectivity with ACC, $r(53) = .32$, $p = .02$, and approaching significance for right amygdala connectivity with ACC, $r(53) = .23$, $p = .09$ (Figure 1). Additional analyses examined group differences in amygdala-ACC connectivity within three age groups (see Supplemental Results).

To evaluate whether the relationship between age and amygdala-ACC connectivity within the anxiety group differed as a function of primary diagnosis, we computed ANCOVAs including the main effects of primary diagnosis and interactions with age. Neither the main effect of primary diagnosis nor the interaction between age and primary diagnosis reached significance in either model ($ps > .35$).

To evaluate whether amygdala-ACC connectivity related to anxiety severity, we computed ANCOVAs examining the main effects of age and anxiety symptoms (i.e., PARS or LSAS) on connectivity. Age interacted with PARS to predict left amygdala-ACC connectivity, $F(1, 65) = 3.85$, $p = .05$, and LSAS to predict right, $F(1, 110) = 6.36$, $p = .01$, and left, $F(1, 110) = 11.18$, $p = .001$, amygdala-ACC connectivity across anxious youth and healthy comparisons (see Supplemental Results). Neither PARS nor LSAS significantly predicted amygdala-ACC connectivity as a main effect or interaction with age within the anxious group only ($ps > .34$).

Left Amygdala-MCG Connectivity—To interpret the 3-way interaction effect of left amygdala-MCG connectivity, the Age x Group interaction was examined for each emotion. The Age x Group interaction was significant for angry faces $F(1, 112) = 5.63$, $p = .02$, such

that age was positively related to left amygdala-MCG connectivity in the anxiety group, $r(53)=.38$, $p<.01$, with no significant association with age for healthy comparisons ($p=.82$). The Age x Group interactions were not significant for fearful or happy faces ($ps>.52$). The main effect of primary diagnosis and its interaction with age were not significant for left amygdala-MCG connectivity during angry face processing ($ps>.46$). The interaction between LSAS and age significantly predicted left amygdala-MCG connectivity across both groups, $F(1, 110)=5.08$, $p=.03$ (see Supplemental Results), but the effect of PARS was not significant and effect of LSAS was not significant within the anxious group only ($ps>.13$).

Left Amygdala-MFG Connectivity—To interpret the Group x Emotion interaction on left amygdala-MFG connectivity, the effect of anxiety group was evaluated for each condition. The anxious group exhibited reduced left amygdala-MFG connectivity compared to healthy participants when processing fearful faces, $F(1, 112)=5.90$, $p=.02$, but the group effect did not reach significance for happy or angry faces ($ps>.09$). The main effect of primary diagnosis was not significant ($p=.75$), and PARS and LSAS were not significantly related to left amygdala-MFG connectivity to fearful faces across groups or within the anxious group ($ps>.08$).

Discussion

We evaluated age-related differences in amygdala connectivity during emotional face processing in youth and young adults with anxiety disorders and healthy comparisons. Our primary finding indicates differential association of age with bilateral amygdala-ACC connectivity in typically developing youth and young adults compared to those with clinically significant anxiety. Healthy comparisons exhibited an age-related shift from positive to negative connectivity of amygdala with ACC during emotional face processing, whereas anxious youth and young adults exhibited an age-related increase in positive amygdala-ACC connectivity.

It has previously been suggested that positive amygdala connectivity with ACC in childhood may indicate greater bottom-up processing and represent immature processing of threat/emotional signals between these regions, with negative connectivity in adulthood indicating more top-down regulation of the amygdala by ACC and improved emotion regulation (17). Our results extend this work and suggest that clinical anxiety is characterized by age-related increases in positive connectivity of amygdala with ACC. Interestingly, rather than a main effect of group, anxiety appeared to be characterized by altered developmental trajectories of amygdala-ACC connectivity. That is, anxious children showed reduced connectivity relative to healthy children, whereas anxious adults showed more positive connectivity compared to the negative relationship between amygdala and ACC observed in healthy adults. Anxiety may be associated with a failure to establish connections between these regions earlier in life and impairments in top-down regulation of the amygdala into young adulthood, and disrupted development of amygdala-ACC connectivity may be a factor contributing to increased risk of anxiety and other psychopathology into adulthood (1).

Consistent with previous work (17), we interpret negative amygdala-ACC connectivity to indicate regulation of the amygdala, such that greater ACC activation to emotional faces

results in adaptive attenuation of amygdala reactivity. It is important to note, however, that because of limitations with measuring timing and direction of associations, we cannot conclude that ACC activates first and down-regulates amygdala. An alternative explanation is that connectivity could indicate signaling from amygdala to ACC or receptiveness of the ACC to inputs from amygdala. For example, in anxiety, increasing positive connectivity across development could indicate greater signaling by amygdala of the need for regulation by the ACC, whereas increasing negative connectivity among healthy comparisons may indicate amygdala signaling for less ACC engagement. It is also possible that more positive amygdala-ACC connectivity in anxious young adults reflects altered cognitive control mechanisms, in which the ACC may upregulate, rather than downregulate, amygdala responses.

We observed abnormal development of amygdala-ACC connectivity in anxiety across both positive (i.e., happy) and negative (i.e., angry and fearful) emotional faces. Previous work on neural processing of emotion in anxiety primarily focuses on threatening faces; thus, findings of altered age-related changes in anxiety across emotion more broadly are noteworthy. Nonetheless, there is some behavioral evidence linking anxiety in youth to deficits in processing of a range of emotional faces (41; 42), and it is possible that in this age range, amygdala connectivity with ACC may underlie processing of salient information rather than specific emotion categories (4). It should also be noted that the emotional faces task included neutral faces on all trials and the task only included three blocks for each emotional face type; thus, it is possible that task design factors limited power to detect valence effects.

In addition to effects of amygdala connectivity with ACC, we found that anxious youth exhibited age-related increases in amygdala connectivity with midcingulate gyrus (MCG) during angry face processing and reduced connectivity between amygdala and middle frontal gyrus (MFG) during processing of fearful faces compared to healthy youth. These effects were not observed with bilateral amygdala connectivity and appeared more sensitive to site/scanner effects than the amygdala-ACC effects (see Supplemental Results). Nonetheless, they suggest that age-related increases in amygdala connectivity extend into MCG during processing of threatening faces in anxious youth. Regions of the MFG have been shown to activate during emotion regulation (43); thus, altered amygdala-MFG connectivity may be one factor contributing to difficulty regulating threat responses in anxiety.

Though we observed distinct patterns of relationships between amygdala connectivity and severity of symptoms depending on age, these effects were apparent across both healthy controls and anxious participants. That is, we did not find evidence that amygdala connectivity related to variation in symptoms within the anxiety group. Future research is needed with larger samples and comparable measures of anxiety severity across the entire sample to further evaluate the extent to which amygdala connectivity with ACC reflects a developmental process that contributes to vulnerability for anxiety disorders or a correlate of symptom severity.

We did not find group differences in amygdala activation to faces. Though there is evidence of heightened amygdala activation to threat in pediatric anxiety (12–16), other work has failed to find effects of anxiety in youth on amygdala reactivity (44) or suggested that amygdala hyperactivation in anxious youth may vary during the course of the task (18). We also did not find effects of age on amygdala activation, which differs from previous evidence of developmental decreases in activation (17; 45), but is consistent with prior findings suggesting that developmental effects are most apparent during passive viewing of emotional faces rather than explicit processing of expressions, as in the current task (46). Though the lack of amygdala activation effects ensures that our connectivity results are less confounded by age or group differences in amygdala activation, it is also problematic for interpreting the meaning of positive compared to negative amygdala-ACC connectivity. Though healthy adults exhibit negative connectivity between amygdala-ACC, this was not associated with an overall decrease in amygdala activation. However, the current findings support previous evidence that amygdala coupling with frontal regions may be a better predictor of individual differences in emotional processing than activation (9; 47).

With regard to limitation, the study is cross-sectional, thus we cannot rule out the possible influence of cohort effects, and future longitudinal research is needed. Second, our power to detect effects of specific diagnoses is limited given the small sample size within each group. In addition, in order to examine a large window of development and include an age range comparable to Gee et al. (17), we combined participants across child and young adult studies and were unable to evaluate effects of dimensional measures of general anxiety severity in the entire sample. Lastly, data were collected across two scanners, and though we controlled for site effects in all analyses and examined our effects separately at each site (see Supplemental Results), we cannot rule out the possibility that scanner differences may have affected the results. Nonetheless, the sample size is large, covering a broad range of development from childhood into young adulthood, and groups were similar on demographics.

This is among the first studies to evaluate age-related changes in amygdala connectivity in clinical anxiety, with results providing insight into how functional development of amygdala connectivity may be disrupted in anxiety and emphasizing the importance of identifying normative developmental pathways in order to better understand the development of psychopathology. Future research is needed to evaluate the extent to which abnormalities in cortico-amygdala function in anxiety are pathologic or compensatory processes and to identify interventions that alter the development of amygdala-ACC connectivity in anxious youth (e.g., increasing positive connectivity in childhood and negative connectivity in early adulthood).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Group x Age interaction on amygdala functional connectivity with ACC during the processing of emotional faces (a). Group x Age interaction on left amygdala-ACC functional connectivity (peak voxel: $-4, 30, 16$; 1016 mm^3 ; shown at $p < 0.005$), and (b) right amygdala-ACC functional connectivity (peak voxel: $2, 34, 14$; 680 mm^3 ; shown at $p < 0.005$); Scatter plots (c) between age and left amygdala-ACC functional connectivity, and (d) between age and right amygdala-ACC functional connectivity for the anxiety group and healthy comparisons.

Table 1

Demographics and current diagnoses for each group.

	Anxiety Disorder (<i>n</i> =57)		Healthy Control (<i>n</i> =61)	
	Mean or %	SD or <i>n</i>	Mean or %	SD or <i>n</i>
Age	17.14	4.81	16.69	5.06
Female	59.6%	34	57.4%	35
Caucasian	56.1%	32	59.0%	36
African American	12.3%	7	14.8%	9
Asian or Pacific Islander	17.5%	10	14.8%	9
Hispanic/Latino	14.0%	8	11.5%	7
Site (scanned at UM)	66.7%	38	57.4%	35
Social anxiety disorder	80.7%	46	0%	0
Generalized anxiety disorder	50.9%	29	0%	0
Separation anxiety	12.3%	7	0%	0
Depressive disorder	5.3%	3	0%	0
Other comorbid anxiety disorder	21.1%	12	0%	0

Table 2

Analysis of covariance (ANCOVAs) of amygdala functional connectivity.

	Brain Region	Brodman Area (BA)	Peak MNI Coordinates (x,y,z)	Z-score	Volume (mm ³)
Left Amygdala Functional Connectivity					
Group x Age x Emotion Interaction	MCG	BA 6, 24, 31	4, -10, 50	3.11	688
Group x Age Interaction	ACC	BA 24	-4, 30, 16	3.06	1016
Group x Emotion Interaction	MFG	BA 8	-22, 32, 50	3.16	1040
Right Amygdala Functional Connectivity					
Group x Age x Emotion Interaction	None				
Group x Age Interaction	ACC	BA 24	2, 34, 14	3.41	680
Group x Emotion Interaction	None				

All regions are significant ($p < 0.05$, AlphaSim corrected) within an a priori frontolimbic mask (threshold=58 voxels). Abbreviations: MNI – Montreal Neurologic Institute; MCG – midcingulate gyrus; ACC – anterior cingulate cortex; MFG – middle frontal gyrus.