Published in final edited form as:

J Am Acad Child Adolesc Psychiatry. 2013 March ; 52(3): 290–299.e2. doi:10.1016/j.jaac.2012.12.010.

Intrinsic Functional Connectivity of Amygdala-Based Networks in Adolescent Generalized Anxiety Disorder

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Abstract

Objective—Generalized anxiety disorder (GAD) typically begins during adolescence and can persist into adulthood. The pathophysiological mechanisms underlying this disorder remain unclear. Recent evidence from resting state functional magnetic resonance imaging (R-fMRI) studies in adults suggests disruptions in amygdala-based circuitry; the present study examines this issue in adolescents with GAD.

Method—Resting state fMRI scans were obtained from 15 adolescents with GAD and 20 adolescents without anxiety who were group matched on age, sex, scanner, and intelligence. Functional connectivity of the centromedial, basolateral, and superficial amygdala subdivisions was compared between groups. We also assessed the relationship between amygdala network dysfunction and anxiety severity.

Results—Adolescents with GAD exhibited disruptions in amygdala-based intrinsic functional connectivity networks that included regions in medial prefrontal cortex, insula, and cerebellum. Positive correlations between anxiety severity scores and amygdala functional connectivity with insula and superior temporal gyrus were also observed within the GAD group. There was some evidence of greater overlap (less differentiation of connectivity patterns) of the right basolateral and centromedial amygdala networks in the adolescents with, relative to those without, GAD.

Conclusions—These findings suggest that adolescents with GAD manifest alterations in amygdala circuits involved in emotion processing, similar to findings in adults. In addition, disruptions were observed in amygdala-based networks involved in fear processing and the coding of interoceptive states.

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Disclosure: Drs. Roy, Fudge, Kelly, Benson, Castellanos, Milham, Pine, and Ernst, and Mr. Perry, Ms. Daniele, and Ms. Carlisi report no biomedical financial interests or potential conflicts of interest.

Keywords

generalized anxiety disorder (GAD); amygdala; intrinsic functional connectivity; functional magnetic resonance imaging (fMRI)

Generalized anxiety disorder (GAD) often begins during adolescence.^{1–3} Studies in adults implicate emotion regulation circuits, but there is a need to extend these models to adolescents^{4–6} to clarify whether adult neural deficits are primary or reflect illness duration. Studies in adults using resting state functional magnetic resonance imaging (R-fMRI) show disruption in amygdala-based circuits associated with elevated anxiety⁷ or a GAD diagnosis.⁸ Such methods have not been applied to adolescents with anxiety disorders. We address this gap by comparing measures of amygdala-based intrinsic functional connectivity (iFC) between adolescents with GAD and healthy comparison subjects.

One challenge to studying amygdala function is its heterogeneity. The amygdala comprises three main nuclear subdivisions: basolateral (BLA), centromedial (CMA), and superficial (SFA). Basic studies find specific connectivities of these subdivisions⁹ that may influence anxiety. For example, the BLA nuclei are instrumental in associative learning processes such as fear conditioning through afferents from subcortical and cortical regions including the prefrontal cortex (PFC).^{10,11} Lesions of the BLA influence vigilance,^{12,13} which is perturbed in GAD.¹⁴ The CMA includes the central nucleus, which receives input from the BLA, and channels this information to autonomic and motor centers in the brainstem, acting as a gateway by which fear responses are generated.^{10,15} CMA lesions increase exploratory behavior, suggesting reduced fear.¹³ Finally SFA nuclei, which include cortices on the medial surface of the amygdala, are intimately associated with the olfactory cortex.¹⁶ In humans, the SFA has been implicated in processing socially relevant information and the modulation of approach-avoidant behavior.¹⁷ These distinct amygdala subdivision connectivity profiles have received support from tractography¹⁸ task-based,¹⁷ and R-fMRI^{8,19} studies of healthy adults.

Recent R-fMRI work shows anxiety-related disruption in amygdala-based networks, particularly those including regions of the PFC. In one study, adults with GAD exhibited reduced differentiation between the BLA and CMA networks compared with healthy adults.⁸ Compared with healthy subjects, and across both BLA and CMA subdivisions, adult patients with GAD exhibited weaker iFC with dorsal anterior cingulate cortex and stronger iFC with dorsolateral PFC, two regions implicated in emotion regulation. However, region-specific group comparisons were not performed for the BLA and CMA separately. Studies of healthy adults also suggest anxiety-related alterations in amygdala–PFC connectivity. For example, elevated state anxiety has been shown to predict weaker negative iFC between amygdala and dorsomedial PFC, and weaker positive iFC between amygdala and ventromedial PFC.⁷ This study did not assess iFC of individual amygdala subdivisions. A study of 291 healthy young adults found greater harm avoidance scores associated with greater negative iFC between CMA and PFC, including ventromedial PFC.²⁰ Harm avoidance scores were also correlated with iFC between SEA and frontal pole, as well as between BLA and temporal and occipital areas. Collectively, these studies in adults implicate perturbed amygdala–PFC iFC in anxiety. Whether these alterations in amygdala iFC are present early in the development of GAD is unknown.

Task-based activation studies provide some evidence, albeit inconsistent, for disrupted amygdala functional connectivity in adolescents with GAD. For example, during an attention task, adolescents with GAD showed weaker task-related functional connectivity between amygdala and ventrolateral PFC than did healthy adolescents.⁵ A study using a

different task failed to find this relationship and instead found stronger task-related amygdala–insula interactions in adolescents with GAD than with healthy adolescents, along with stronger amygdala–posterior cortical connectivity⁶ Together, these studies suggest GAD-related alterations in the functional co-activation of amygdala and cortical regions during emotional tasks.

The current study uses R-fMRI to examine the integrity of amygdala-based networks in adolescent GAD, interrogating each amygdala subdivision, BLA, CMA, and SEA. Based on prior findings, we hypothesize perturbation of amygdala–PFC iFC; however, whole-brain analyses are used to explore other regions of disrupted connectivity. For regions showing significant group differences, we evaluate relationships between amygdala iFC and anxiety severity. Because greater overlap of CMA and BLA networks was found in adults with GAD relative to healthy adults,⁸ we also examine the distinctiveness of these two networks to test whether adolescents with GAD similarly show elevated network overlap, reflecting reduced subregional functional specialization.

METHOD

An advantage of R-fMRI is that although scanner- and site-related variation is detectable, robust effects associated with phenotypic variables such as diagnosis are discernible when data from multiple sites are combined.^{21–24} We exploit this robustness to combine datasets from two sites to attain sufficiently large samples of medication-free pediatric participants.

Participants (12–17 years of age) were recruited across two sites, the New York University (NYU) Child Study Center, and the National Institute of Mental Health (NIMH). Fifteen adolescents with a diagnosis of GAD (NYU: $n = 9$; NIMH: $n = 6$) and 20 age-, sex-, IQ-, and scanner-matched healthy comparison subjects without anxiety underwent scanning (NYU: $n = 13$; NIMH: $n = 7$) (Table 1). Across groups, participants were excluded if they had a history of trauma, pervasive developmental disorder, psychosis, current or past use of psychotropic medication, or $IQ < 70$. Participants were not engaged in any formal treatment at the time of the study. All participants were right handed. The study was approved by the institutional review boards of the NYU School of Medicine, New York University, and the National Institute of Mental Health. Informed consent was obtained from parents, and assent was obtained from participants before participation.

Diagnoses were determined using the Kiddie Schedule for Affective Disorders–Present and Lifetime version (K-SADS-PL).²⁵ At both sites, interviewers were trained to criterion (minimum 80% agreement) by experienced clinicians and were regularly monitored by these clinicians through in-person meetings with both interviewers and potential research participants. Prior collaboration between these sites generated expected findings on the correlates of anxiety,²⁶ thus supporting the use of similar combined samples in the present study. Within the GAD group, 12 adolescents (75%) received a secondary comorbid diagnosis (Table 1), as is typical in pediatric GAD.^{27–28} Healthy comparison subjects were also assessed using the K-SADS-PL, and did not qualify for any psychiatric diagnoses. Parent and child versions of the Screen for Child Anxiety Related Emotional Disorders (SCARED)²⁹ were administered to both patients and comparison subjects as dimensional measures of anxiety symptoms.

Data Acquisition and Image Processing

Imaging data were acquired on three 3T scanners: a Siemens Allegra (NYU; 13 healthy subjects, 9 GAD), a General Electric (NIMH; three healthy subjects, three GAD), and a Signa (NIMH; four healthy subjects, three GAD). Participants were instructed to remain still with eyes open while a black screen with a central white crosshair was placed in their field

of view. The R-fMRI sequence consisted of a 6-minute acquisition of 180 echo-planar imaging (EPI) functional volumes (repetition time [TR] = 2000 milliseconds; echo time [TE] = 30 milliseconds; flip angle = 90; field-of-view [FOV] = 240 × 192 mm; acquisition voxel size 3×3×4 mm). T1-weighted anatomical images were obtained for spatial normalization and localization purposes.

All data were preprocessed using AFNI (<http://afni.nimh.nih.gov/afni/>) and FSL (<http://www.fmrib.ac.uk/fsl/>), as in previous studies.³⁰ Participants with R-fMRI scans with a maximum displacement greater than 3.1 mm were excluded from further analyses. Linear registration to the Montreal Neurological Institute (MNI) 152 template was performed using Functional Magnetic Resonance Imaging of the Brain (FMRIB) Linear Image Registration Tool (FLIRT); the FMRIB Nonlinear Image Registration Tool (FNIRT) was used to perform nonlinear registration. Preprocessed data were regressed on nine nuisance covariates, removing variance associated with signals derived from white matter, cerebrospinal fluid, the global signal, and six motion parameters.

Time Series Extraction

Mean time series were extracted bilaterally for the three amygdala regions-of-interest (ROIs).¹⁹ Standardized amygdala ROIs were defined using the Juelich histological atlas implemented in FSL, which defines basolateral (BLA), centromedial (CMA), and superficial (SFA) subdivisions based on stereotaxic, probabilistic maps of cytoarchitectonic boundaries (see Figure SI, available online).³¹ Based on this atlas, the BLA (left: 1,840 mm³; right: 1,920 mm³) comprises the basomedial, basolateral, paralaminar, and lateral nuclei, the CMA (left: 176 mm³; right: 224 mm³) comprises the medial and central nuclei, and the SFA (left: 952 mm³; right: 760 mm³) comprises of the posterior and ventral cortical nuclei, the anterior amygdaloid area, the amygdala-hippocampal area, and the amygdala-pyriform transition area. Each ROI included voxels that exhibited at least 50% probability of belonging to their given subdivision, with overlapping voxels delegated to the region with highest probability. Mean time series were created by averaging across the time series of all voxels in each ROI.

Data Analysis

Voxelwise Examination of Group Differences in Amygdala iFC—For each participant, first-level multiple regression analyses were conducted using the FMRIB Improved Linear Model (FILM) in FSL. For each hemisphere, a multiple regression model General Linear Model was created that included time series predictors for all three subdivisions. This resulted in individual subject-level maps of all voxels exhibiting positive or negative iFC with each amygdala subdivision regressor, accounting for the relationships of the other subdivisions.

Group-level analyses were carried out using a random-effects, ordinary least-squares model that included two group mean predictors (one for each group) and three demeaned covariates (age, sex, and scanner) for each group. Group comparisons were conducted using cluster-level Gaussian Random Field theory for multiple comparison correction, with additional correction for the inclusion of three amygdala ROIs in each model ($Z > 2.3$; $p < .05/3$, corrected). Further examination of amygdala iFC in the regions exhibiting significant group differences was conducted using SPSS 19.0 (SPSS Inc., Chicago, IL). Spherical binary masks (4-mm radius) were created around the maximum peak for each significant cluster. Average partial regression coefficients (i.e., iFC) were extracted for each of these ROIs for each participant. One-sample *t* tests were used to determine whether measures of iFC differed significantly from zero.

Examination of Comorbidity Effects—Secondary analyses examined the effects of comorbidity. Using the average partial regression coefficients extracted for each of the significant ROIs obtained from the analyses described above, two multivariate analyses of variance (ANOVAs) were conducted to compare adolescents with GAD (excluding either those with major depressive disorder [MDD] or those with social phobia) to healthy adolescents.

Relationship between iFC and Anxiety Severity—As in prior studies,³² total scores from the Parent and Child SCARED were averaged to derive a single index (SCARED-PC). Correlational analyses were conducted between data extracted for each of the significant ROIs (as described above) and SCARED-PC scores, both in the sample as a whole and separately in the patients and healthy comparison subjects.

Differentiation of Basolateral and Centromedial Amygdala iFC—To examine group differences in the differentiation of BLA and CMA functional networks, we conducted a second set of analyses following Etkin *et al.*⁸ First, we established regions involved in BLA- and CMA-based functional connectivity networks from group analyses for healthy comparison subjects, yielding eight sets of results (positive and negative for the two subdivisions in each hemisphere). When results yielded more than one cluster, these clusters were combined into a single cluster representing all regions of the network. A mask was created for each of these eight clusters and average partial regression coefficients were extracted for each of these clusters from the CMA-based and BLA-based regression analyses. This resulted in four iFC values per amygdala ROI, per hemisphere, as in the following examples: positive iFC of the right BLA with typical right BLA targets (own network); positive iFC of the right BLA with typical right CMA targets (other network); negative iFC of the right BLA with typical right BLA targets (own network); and negative iFC of the right BLA with typical right CMA targets (other network). These data were analyzed using four 2 (GAD versus comparison) $\times$ 2 (CMA vs. BLA) $\times$ 2 (own versus other) ANOVAs implemented in SPSS (left, right, positive, negative). A three-way interaction would suggest group differences in the differentiation of the iFC of the two subdivisions.

RESULTS

As shown in Table 1, no group differences were observed for sex, age, IQ, race/ethnicity, or movement (maximum displacement) during the resting state scan, or the percentage of participants from each site. Scores on the SCARED-PC were significantly higher for the GAD group than the healthy comparison group ($t_{32} = 8.2$, $p < .001$).

Voxelwise Group Differences

Centromedial Amygdala (CMA)—Adolescents with GAD showed stronger negative iFC in a cluster of voxels in ventromedial PFC (VMPFC) including pregenual and subgenual anterior cingulate, extending subcortically to striatal regions (caudate nucleus and nucleus accumbens) (Figure 1 and see Table S1, available online, for complete results). As shown in Table 2, mean iFC between right CMA and these regions was significantly negative in adolescents with GAD and significantly positive in healthy comparison subjects. A second cluster including insula and superior temporal gyrus also exhibited group differences in right CMA iFC; adolescents with GAD exhibited stronger positive iFC than healthy comparison subjects (Figure 1).

Significant differences were also observed between left CMA and a left frontal-pole cluster encompassing ventrolateral PFC (VLPFC; Figure 1 and see Table S1, available online). In adolescents with GAD, mean iFC between these regions did not differ significantly from

zero, whereas in healthy comparison adolescents the correlation was significantly negative (Table 2).

Superficial Amygdala (SFA)—Group differences were observed for iFC between right SFA and a cluster extending from dorsomedial to dorsolateral PFC. adolescents with GAD exhibited positive iFC while healthy comparison subjects exhibited negative (Figure 2 and Table 2). A second cluster encompassing regions in the cerebellum and brainstem showed significantly greater negative iFC in adolescents with GAD than comparison subjects. Specifically, adolescents with GAD exhibited negative iFC with right SFA; no significant iFC was observed between these regions and right SFA in healthy comparison adolescents (Figure 2 and Table 2). No group differences were observed for the left SFA.

Basolateral Amygdala (BLA)—Group differences were observed in iFC between the left BLA and a cluster in brainstem extending to cerebellum (Figure 2 and see Table S1, available online). Adolescents with GAD showed significant positive iFC; healthy adolescents showed no significant iFC (Table 2). No group differences were observed for the right BLA.

Effects of Comorbidity

Group differences were examined further in two additional analyses. The first excluded the adolescents with GAD with a comorbid diagnosis of MDD ($n = 3$). Group differences in iFC remained significant across all amygdala regions ($F_{8/23} = 7.65$; $p < .001$). The second analysis excluded the adolescents with GAD with a comorbid diagnosis of social phobia ($n = 5$). Again, the multivariate ANOVA remained significant ($F_{8,21} = 7.11$, $p < .001$).

Amygdala iFC and Anxiety Severity

In the sample as a whole, iFC measures of all regions exhibiting between-group differences were correlated with SCARED-PC scores ($r = \pm 0.48$ to ± 0.65 ; all $p < .005$). For the GAD group alone, a strong positive correlation was observed between SCARED-PC scores and iFC of the right CMA with the cluster including insula and superior temporal gyrus ($r = 0.58$, $p = .02$) (see Figure S2, available online). For the healthy comparison group alone, no correlations were observed between iFC and SCARED-PC scores.

Differentiation of CAM and BLA iFC

Mixed-effects ANOVAs were conducted to assess group differences in the differentiation of iFC between the CMA and BLA subdivisions, per Etkin *et al.*⁸ A significant group $\times$ amygdala ROI $\times$ target (own versus other) interaction was observed for regions of positive iFC for right CMA and BLA ($F_{1,33} = 5.99$, $p = .05$, partial $\eta^2 = 0.12$). Follow-up analyses show a significant group $\times$ target interaction for targets of the right BLA ($F_{1,33} = 4.38$, $p = .04$), but no interaction for right CMA targets. As shown in Figure 3, adolescents with GAD showed stronger iFC between the right CMA and targets of the right BLA compared with the healthy comparison group ($t_{33} = -2.04$, $p < .05$). None of the other analyses (left hemisphere, negative iFC) were significant.

DISCUSSION

Study findings have revealed alterations in iFC of individual amygdala subdivisions with prefrontal regions, consistent with emotion regulation models of GAD.³³ Additional iFC group differences were observed in the striatum, insula, superior temporal gyrus, brainstem, and cerebellum, suggesting more widespread disruption of amygdala networks in adolescent GAD than observed previously in task-based studies. We discuss these findings in terms of specific cognitive processes likely involved in GAD: disruptions of amygdala–PFC iFC that

imply alterations of emotion evaluation and regulation; disruptions of amygdala–insula iFC that suggest dysfunction in the integration of interoceptive information; and disruptions of amygdala–cerebellum iFC that may reflect disruption of fear learning. In addition, we found similar reduced distinctness between CMA and BLA networks, as reported in adults.

Disruption of Amygdala–PFC iFC

Group differences were observed in the iFC of amygdala with medial prefrontal regions involved in emotion processing and regulation.^{34–37} Adolescents with GAD exhibited negative iFC between right CMA and VMPFC, whereas healthy adolescents exhibited positive iFC between these regions. Conversely, iFC between the SFA and dorsomedial PFC (DMPFC) was positive in adolescents with GAD and negative in healthy comparison subjects. This is consistent with adult data from iFC,⁷ task-based,³⁸ and tractography³⁹ studies suggesting that disruptions seen in anxious adults are already present in adolescents with GAD. Recent theories suggest that DMPFC and VMPFC serve separable emotion regulation functions that likely emerge from distinct relationships with the amygdala.^{37,40} Specifically, VMPFC, including the subgenual anterior cingulate cortex (ACC), is implicated in automatic emotion regulation and is essential to successful extinction of fear responses through connections with the amygdala.^{37,41–3} The DMPFC has been implicated in appraisal and cognitive regulation of emotion.^{37,40} Here, adolescents with GAD exhibited alterations in both amygdala-based networks, suggesting disruption of multiple emotion regulation processes.

Group differences were also observed in amygdala iFC with lateral PFC regions implicated in emotion regulation.^{40,44,45} Adolescents with GAD showed positive iFC between right SFA and right DLPFC, whereas healthy comparison subjects showed negative iFC in this circuit. This latter result is consistent with previous findings in adults.¹⁹ In addition, iFC between left CMA and VLPFC was less negative in adolescents with GAD than in healthy comparison subjects, similar to previous task-based findings.⁵ Moreover, a recent study showed that, compared with low-anxious adults, high-anxious adults exhibit greater activity in lateral PFC regions when down-regulating emotions, suggesting a need for more effortful emotional control.⁴⁶ Thus, disruptions in typical iFC between amygdala and lateral PFC further support the need to examine emotion regulation processes in adolescents with GAD.

Disruption of Amygdala–Insula iFC

Adolescents with GAD exhibited greater positive iFC than healthy comparison subjects between right CMA and a cluster including the insula, which replicates previous task-based work.⁶ The insula is involved in perception of interoceptive states and integrates such information with salient environmental inputs through bidirectional amygdala connections.⁴⁷ Recent theory suggests that these processes are disrupted in anxious individuals who cannot easily differentiate typical fluctuations in interoceptive stimuli from potentially aversive body signals, leading to increased worry.⁴⁸ Consistent with this, evidence has shown insula activation in response to worry in adults with GAD⁴⁹ and altered insular responses associated with intolerance of uncertainty, which is implicated in the etiology and maintenance of GAD.⁵⁰

Disruption of Amygdala–Cerebellum iFC

A complex pattern of group differences emerged for iFC between amygdala and cerebellum. For the left BLA, adolescents with GAD exhibited positive iFC with the cerebellum, whereas no significant iFC was observed in comparison subjects. Conversely, iFC between the right SFA and cerebellum was negative in the GAD group, whereas the comparison group showed no significant relationship. Although these results suggest GAD-related disruptions in amygdala–cerebellar iFC, we note the absence of significant iFC between the

amygdala and cerebellum in healthy comparison subjects. This was surprising, given previous findings showing significant amygdala–cerebellar iFC in adults.^{8,19} Further work should examine putative developmental changes in amygdala–cerebellar iFC, particularly in light of the cerebellum’s protracted developmental course that continues through adolescence.⁵¹ Basic research supports anatomical connectivity between the cerebellar vermis and amygdala,^{52–54} and evidence from human and animal studies indicates a primary role of the cerebellum in fear expression and memory.^{54–56} Thus, together with the altered findings of CMA-subgenual ACC iFC, alterations in amygdala–cerebellar iFC in adolescents with GAD suggest disruptions in normative fear processing, although confirmation of such deficits awaits further study.

Anxiety Severity and Amygdala iFC

As expected based on the group differences observed, amygdala iFC measures were significantly correlated with anxiety severity across the whole sample. Thus, within-group correlations are more meaningful measures of a dimensional association between anxiety and amygdala iFC. In the GAD group, only amygdala–insula iFC was correlated with SCARED-PC scores, further supporting observed group differences. No significant correlations were observed for the healthy adolescents, which is likely the result of the restricted range of scores in this group.

Distinct iFC Connectivity Patterns between the CMA and BLA

Finally, to inquire whether disruptions in iFC of amygdalar subdivisions in adults with GAD are also present in adolescents, we directly compared CMA and BLA networks. Positive iFC of the right CMA with BLA targets was greater in adolescents with GAD than healthy comparison subjects, suggesting greater overlap of function between these two subdivisions, or less distinct functional specialization. This is consistent with prior findings,⁸ although adults with GAD also showed alterations in differentiation across hemispheres and with iFC of the BLA with CMA targets, which we did not find. Differences between our results and previous adult findings may reflect developmental differences in amygdala organization that remain to be confirmed in larger samples.

Our findings should be considered in light of limitations. First, statistical power was limited by relatively small sample sizes, although our sample sizes are comparable to those in neuroimaging studies of GAD in adults^{8,39,57} and adolescents.^{4,6} Limited sample sizes are especially problematic for fMRI studies of clinical populations, because of factors such as low prevalence, increased logistical and financial challenges related to recruitment and phenotypic assessment, and behavioral limitations precluding participation in the MR environment (e.g., anxiety). Importantly, we highlight the potential utility of pooling data from independent sites. Second, our findings provide only a first step for such multi-site studies. Additional steps would address our limited ability to quantitatively assess the cross-site reliability of iFC measures. For example, future efforts will need to incorporate techniques for cross-site calibration, such as the use of phantoms and possibly even shared subjects. Third, the GAD group was heterogeneous in terms of diagnostic comorbidity. Such comorbidity is typical of GAD in adolescence²⁸; a sample of adolescents with GAD without comorbidity would lack external validity, and post hoc analyses showed little impact of comorbid major depressive disorder or social phobia on our findings. Finally, future work will need to include adolescents with a non-GAD anxiety disorder to test the specificity of our findings, and could include assessment of psychopathology in first-degree relatives to test for differences related to familiarity.

This is the first study to use voxelwise comparisons of GAD patients and healthy comparison subjects for each of three amygdala subdivisions, across positive and negative

iFC networks. Findings support hypotheses that adolescents with GAD exhibit alterations in amygdala circuits underlying emotional processes known to be deficient in GAD in adults, but also suggest perturbations in circuits involved in fear learning and processing interoceptive states. Thus, these results have the potential to inform broader pathophysiological models of GAD across development. Studies of adults with GAD using similar methodologies, and of amygdala iFC across typical development, are needed to disentangle putative differential as well as interactive effects of developmental and pathological processes. &

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The project described was supported by NIMH Career Development Award K23 MH074821 (A.K.R.).

The authors thank the participants and their families as well as Rachel Chizkov, B.A., of the New York University Child Study Center for her assistance in assembling the necessary datasets.

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FIGURE 1.

Regions showing group differences in functional connectivity (iFC) with the centromedial amygdala (CMA). Note: Scatterplots are shown for each significant cluster. White indicates National Institute of Mental Health (NIMH) Scanner #1; black indicates NIMH Scanner #2; Gray indicates New York University (NYU) scanner. GAD = generalized anxiety disorder.

FIGURE 2.

Regions showing group differences in functional connectivity (iFC) with superficial (SFA) and basolateral (BLA) amygdala. Note: Scatterplots are shown for each significant cluster. White indicates National Institute of Mental Health (NIMH) scanner #1; Black indicates NIMH scanner #2; Gray indicates New York University (NYU) scanner. GAD = generalized anxiety disorder.

FIGURE 3.

Differential functional connectivity (iFC) between the centromedial amygdala and regions of the centromedial (CMA) and basolateral (BLA) networks observed in the healthy comparison group. Note: Blue represents healthy comparison subjects; red represents adolescents with generalized anxiety disorder. * $p < .05$.

TABLE 1

Demographic and Diagnostic Characteristics

Variable	GAD Patients (n = 15)	Healthy Subjects (n = 20)
Mean age (SD)	14.9 (1.7)	14.8 (1.7)
Sex (female), n (%)	10 (66.7)	13 (65)
Race/ethnicity, n (%)		
White	9 (60)	5 (25)
African American	2 (13.3)	7 (35)
Asian	2 (13.3)	2 (10)
Hispanic	1 (6.7)	3 (15)
Other	1	1 (5)
Mixed	0	4 (20)
Mean estimated IQ (SD)	109.5(8.5)	106.6 (15) ^a
Site, n (%)		
NYU	9 (60)	13 (65)
NIMH	6 (40)	7 (35)
Mean maximum displacement (SD)	1.6 mm (.99)	1.1 mm (.91)
Mean SCARED-PC (SD)	30.3 (12.9)	5.6 (2.9)
Anxiety diagnosis		
GAD	15	0
Social phobia	5	0
Specific phobia	2	0
Panic disorder	1	0
OCD	1	0
SAD	2	0
Other diagnosis		
MDD	3	0
ADHD	1	0

Note: ADHD = attention-deficit/hyperactivity disorder; GAD = generalized anxiety disorder; MDD = major depressive disorder; NIMH = National Institute of Mental Health; NYU = New York University; OCD = obsessive-compulsive disorder; SAD = separation anxiety disorder; SCARED-PC = Screen for Child Anxiety Related Emotional Disorders Parent and Child Composite Score.

^aIQ data missing for one healthy comparison subject.

TABLE 2

Group Differences in Functional Connectivity of Amygdala Subdivisions

Amygdala Region	Target	X	Y	Z	One-Sample T-Test Results ^a
Centromedial (R)					
HC > GAD	Subgenual ACC	28	38	-10	GAD: $t = -3.39, p = .004$ HC: $t = 3.65, p = .002$
GAD > HC	Insula, Superior Temporal Gyrus	-36	-24	2	GAD: $t = 6.17, p < .001$ HC: $t = 2.69, p = .015$
Centromedial (L)					
GAD > HC	Ventrolateral PFC	-20	34	6	GAD: $t = 1.31, p = .19$ HC: $t = -6.07, p < .001$
Superficial (R)					
HC > GAD	Brainstem, cerebellum	0	-34	-40	GAD: $t = -2.16, p = .05$ HC: $t = 1.76, p = .09$
GAD > HC	Medial PFC	10	28	44	GAD: $t = 3.79, p = .002$ HC: $t = -3.70, p = .002$
Basolateral (L)					
GAD > HC	Brainstem, cerebellum	8	-34	-36	GAD: $t = 3.53, p = .003$ HC: $t = 1.61, p = .11$

Note: ACC = anterior cingulate cortex; L = left hemisphere; NS = not significant; PFC = prefrontal cortex; R = right hemisphere.

^aFourteen degrees of freedom for generalized anxiety disorder (GAD) group and 19 degrees of freedom for healthy comparison (HC) group.