Cortical Thickness, Cortico-Amygdalar Networks, and Externalizing Behaviors in Healthy Children

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Background: Fronto-amygdalar networks are implicated in childhood psychiatric disorders characterized by high rates of externalizing (aggressive, noncompliant, oppositional) behavior. Although externalizing behaviors are distributed continuously across clinical and nonclinical samples, little is known about how brain variations may confer risk for problematic behavior. Here, we studied cortical thickness, amygdala volume, and cortico-amygdalar network correlates of externalizing behavior in a large sample of healthy children.

Methods: Two hundred ninety-seven healthy children (6–18 years; mean = 12 ± 3 years), with 517 magnetic resonance imaging scans, from the National Institutes of Health Magnetic Resonance Imaging Study of Normal Brain Development, were studied. Relationships between externalizing behaviors (measured with the Child Behavior Checklist) and cortical thickness, amygdala volume, and cortico-amygdalar structural networks were examined using first-order linear mixed-effects models, after controlling for age, sex, scanner, and total brain volume. Results significant at $p \leq .05$, following multiple comparison correction, are reported.

Results: Left orbitofrontal, right retrosplenial cingulate, and medial temporal cortex thickness were negatively correlated with externalizing behaviors. Although amygdala volume alone was not correlated with externalizing behaviors, an orbitofrontal cortex-amygdala network predicted rates of externalizing behavior. Children with lower levels of externalizing behaviors exhibited positive correlations between orbitofrontal cortex and amygdala structure, while these regions were not correlated in children with higher levels of externalizing behavior.

Conclusions: Our findings identify key cortical nodes in frontal, cingulate, and temporal cortex associated with externalizing behaviors in children; and indicate that orbitofrontal-amygdala network properties may influence externalizing behaviors, along a continuum and across healthy and clinical samples.

Key Words: Amygdala, cortical thickness, externalizing behavior, healthy children and adolescents, network, structural magnetic resonance imaging

Externalizing behaviors (e.g., aggressive, noncompliant, oppositional) are common in children and adolescents (1). Problems within this behavioral realm represent a leading cause for referral to childhood mental health services (2). Severe and persistent externalizing behaviors are characteristic of externalizing disorders, such as oppositional defiant disorder, conduct disorder, and attention-deficit/hyperactivity disorder (ADHD) (3). These common psychiatric disorders begin in early life and are associated with substantial social and financial costs, with ongoing effects into adulthood (4,5).

Neuroimaging and brain lesion studies demonstrate structural and functional correlates of externalizing behaviors in healthy and clinical samples within amygdalae, medial prefrontal (including orbital and medial frontal) cortex, and cingulate cortex (6–9). In particular, structural and functional differences within a fronto-amygdalar circuit, comprised of amygdalae, orbital/medial frontal cortex, and white matter pathways linking these regions, have been highlighted in patients with psychiatric disorders characterized by high rates of externalizing behaviors versus control subjects (10), including in children and adolescents (11–13). Animal connectivity studies demonstrating the presence of direct inputs from medial prefrontal cortex onto amygdalar inhibitory connections point to 1) the role of medial prefrontal cortex in top-down control over amygdala activation, and 2) the importance of this circuitry in emotion regulation (14). Recent functional magnetic resonance imaging (fMRI) findings showing reduced functional connectivity between orbitofrontal cortex (OFC) and amygdala in externalizing disordered youths, versus control subjects, support the hypothesis that impaired OFC-mediated regulatory control over amygdala may be an etiopathogenic factor in externalizing disorders (13). However, neuroimaging studies to date have mainly focused on small clinical samples, often in the presence of comorbid psychiatric conditions and medication exposure that may separately affect brain structure and function. Further, the National Institute of Mental Health has recently initiated the Research Domain Criteria project, calling for a shift in research focus from categorical diagnoses toward examination of symptom dimensions, citing evidence that mental disorders stem from alterations in brain circuits that confer risk for illness symptoms (15). As externalizing behaviors are distributed

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continuously across clinical and nonclinical samples (1), a dimensional (as opposed to categorical) approach to relating brain and behavior in healthy children may provide novel insights into how variations in neural circuits influence behavior expression, while avoiding confounding factors present in clinical samples.

In the present study, we used a large longitudinal sample from the National Institutes of Health (NIH) Magnetic Resonance Imaging (MRI) Study of Normal Brain Development (16) to examine 1) the relation between cortical thickness (at over 80,000 vertices across the cerebral cortex) and externalizing behaviors, measured with the Child Behavior Checklist (CBCL); 2) the relation between amygdala volume and externalizing behaviors; and 3) whether structural correlations between amygdala volume and cortical thickness predict externalizing behaviors in children. We hypothesized that 1) amygdala volume and medial prefrontal and cingulate cortex thickness would correlate with externalizing behaviors; and 2) network-based analyses would uncover relations between fronto-amygdalar circuit structure and externalizing behavior that would mirror previous connectivity findings in externalizing disorder youths.

Methods and Materials

Sample

The NIH MRI Study of Normal Brain Development is a multisite project providing a demographically representative and normative sample aimed at characterizing healthy brain maturation in relationship to behavior (16,17). Subjects were recruited at six study centers across the United States. Continuous monitoring at all sites ensured recruitment of participants that were demographically representative of the US population (based on age, sex, ethnicity, and socioeconomic status). Informed consent from parents and child assent from participants were obtained. The NIH MRI Study of Normal Brain Development's objective 1 database (release 4.0) used for this study included 431 children (4:6–18:3 years) who underwent repeated cognitive, neuropsychological, and behavioral testing and MRI brain scanning, performed at 2-year intervals for up to three research study visits (i.e., data acquired from participants at one, two, or up to three time points). As this dataset was acquired to study developmentally healthy children, exclusion criteria included IQ <70; any CBCL subscale score ≥ 70; first-degree relative with genetically related mental retardation, schizophrenia, alcohol dependence, bipolar disorder, obsessive-compulsive disorder, Tourette syndrome, recurrent/chronic major depressive disorder, pervasive developmental disorder, or attention-deficit/hyperactivity disorder; or personal history of significant closed head injury or medical/neurological disorder, abnormal neurological exam, pregnancy/birth-related/prenatal adverse event, language disorder, substance use, or Axis I psychiatric disorder assessed using the Diagnostic Interview Schedule for Children (DISC) (18), except enuresis, encopresis, nicotine dependency, specific phobia, social phobia, and adjustment or oppositional defiant disorder (ODD). Structural MRI and clinical/behavioral data were consolidated and analyzed within a purpose-built database at the Montreal Neurological Institute (MNI) (McGill University, Montreal, Canada). Institutional Review Boards at each center approved all study protocols and procedures (Supplement 1).

Child Behavior Checklist

For all study participants, externalizing behaviors were characterized at each study visit using the CBCL, an age-appropriate standardized questionnaire, with good psychometric properties, designed for completion by the parent (1). The CBCL is divided into eight subscales; subscale *t* scores ≥ 70 signify clinically significant symptoms. By combining delinquent and aggressive behavior subscales, the CBCL can provide a raw externalizing score, yielding a noncategorical assessment of externalizing behavior problems, with lower scores signifying the presence of few externalizing behaviors and *t* scores >63 signifying clinically significant symptoms (19). The CBCL externalizing score has demonstrated excellent agreement with clinical diagnoses of externalizing disorders (20). Here, we included participants whose parents completed the CBCL for children aged 6 to 18 years at each visit, yielding up to three sets of CBCL scores per participant. Although no subject had clinically significant externalizing symptoms (score >63) or an exclusionary Axis I disorder at the time of study enrollment, participants were kept in the study if a clinically significant CBCL score or Axis I disorder was present upon longitudinal follow-up. In our sample, 3 of the 297 participants had externalizing *t* scores within the clinical range (scores = 64–69) when the CBCL was repeated after initial study enrollment. Two additional study participants met DSM-IV-Text Revision criteria for an externalizing disorder during the study period (i.e., one with ODD on study enrollment and one 14-year-old male with conduct disorder on longitudinal follow-up). For these two participants, all CBCL subscale and externalizing *t* scores were in the nonclinical range, across longitudinal follow-up, suggesting that any symptoms that were present were of low clinical severity.

MRI Protocol

Structural magnetic resonance (MR) images were acquired on 1.5T scanners; 1 mm in-plane resolution ($\sim$1.5 mm slice thickness was allowed for GE scanners to accommodate for this scanner's 124-slice limit), whole-brain coverage, and multiple contrasts (T1-weighted, T2-weighted, and proton density-weighted) were obtained. A three-dimensional T1-weighted spoiled gradient recalled echo sequence was selected. Intersite reliability of brain structure measurements was monitored using the American College of Radiology Phantom and regular scanning of a living phantom. All data processing took place at the MNI (16). See Supplement 1 for MRI quality-control procedures.

Automated Image Processing

Quality-controlled native MR images were processed through the CIVET pipeline (version 1.1.11; developed at the MNI, Montreal, Canada) (21). Native MR images were linearly registered to a standardized MNI-Talairach space, based on the International Consortium of Brain Mapping (ICBM)-152 dataset (MNI) (22), inhomogeneity corrected (23), and tissue classified. The CLASP algorithm (MNI) was used to extract inner (gray/white matter) and outer (pial) cortical surfaces for generating cortical thickness measurements at 40,962 vertices per hemisphere (24,25). Cortical surfaces were nonlinearly registered to an average surface to establish vertex correspondence between subjects. Subsequently, a reverse linear transformation to native image space was performed to estimate cortical thickness at each cortical point using the *t* link metric (representing distance between outer and inner cortical surfaces) (26). Blurring along the cortical surface was undertaken using a 20 mm surface-based diffusion blurring kernel. This kernel size closely approximates values that have been previously recommended to optimize signal-to-noise ratio for cortical thickness analysis using the CIVET pipeline (26,27). Regional volumes for amygdalae were obtained using a validated, fully automatic label-fusion based segmentation method using

in-house software developed at the MNI, yielding an optimal median Dice Kappa of .826 and Jaccard similarity of .703 for amygdalae (28). A visual quality control (blinded to participants' externalizing score) of native cortical thickness images was implemented to ensure that values were in an appropriate range for all included participants (29).

Handedness

A measure of hand preference was adapted from the Edinburgh Handedness Inventory (30). Hand preference was determined based on seven of eight responses with the same hand (17).

Data Analysis

Analysis 1. Cortical Thickness and Externalizing Behavior. Using CBCL and MRI measures acquired for each participant at each time point (i.e., longitudinal data), absolute native-space cortical thickness was regressed against raw CBCL-derived externalizing scores at each cortical thickness sampling point across the cortex, after controlling for age, sex, scanner, and total brain volume, using a first-order linear mixed-effects model. A mixed-effects model was chosen to enable analysis of all unbalanced longitudinal data (i.e., acquired from participants at one, two, or up to three time points), while accounting for within-subject and between-subject variance and maximizing statistical power (31–33) (Supplement 1). Handedness and full-scale IQ [measured using the Wechsler Abbreviated Scale of Intelligence (34)] were tested as potential confounding variables but were removed as these variables did not contribute significantly to results. A second-order regression model was tested to examine for a quadratic association between externalizing score and cortical thickness. Age was modeled as a first-order linear effect (in contrast to quadratic or cubic) as previous work on this sample has shown that age effects on cortical thickness are best described by a first-order linear function (35,36). Exploratory analyses of interaction effects for age or sex by externalizing score were conducted. To account for multiple comparisons, whole-brain correction using random field theory (RFT), set at $p \leq .05$, was applied (37).

Analysis 2. Amygdala Volume and Externalizing Behavior. Using CBCL and MRI measures acquired for each participant at each time point (i.e., longitudinal data), externalizing score was linearly regressed against left or right amygdala volume, using a mixed-effects model, after controlling for age, sex, scanner, and total brain volume, using R 2.15.2 software (http://www.R-project.org) (38). Interaction effects for age or sex by externalizing score were examined. A Bonferroni correction was applied to correct for comparisons of left and right amygdala volume ($\alpha = .05/2$, $p \leq .025$).

Analysis 3. Cortico-Amygdalar Networks and Externalizing Behavior. To test for the presence of cortico-amygdalar networks underlying externalizing behavior using our dimensional data, we entered longitudinally acquired amygdala volume measurements (i.e., acquired for each participant from up to three time points) to our linear mixed-effects model (Analysis 1). Separate analyses adding left or right amygdala volume-by-externalizing score interaction terms were carried out. Triple interaction effects for age or sex by externalizing score by left or right amygdala volume were also examined. Where our dimensional analyses were significant and interaction effects were found, follow-up linear regression analyses were then completed after dividing our sample into low and high externalizing groups, to aid in the interpretation of primary cortico-amygdalar network findings. For this follow-up analysis, externalizing groups were defined based on median values for raw externalizing scores (median score = 2, low externalizing group, score = 0–2 [$n = 280$], high externalizing group, score ≥ 3, [$n = 212$]). Statistical analyses examining cortical thickness relationships with externalizing behavior were implemented using SurfStat, a statistical toolbox created for MATLAB 7 (The MathWorks, Inc., Natick, Massachusetts) at the MNI (http://www.math.mcgill.ca/keith/surfstat/) (37).

Post Hoc Analyses

Externalizing scores often correlate highly with measures of inattention and hyperactivity/impulsivity (39). In the present sample, externalizing scores were significantly correlated with the CBCL-derived attention problem subscale and Diagnostic and Statistical Manual of Mental Disorders oriented ADHD scale scores (Pearson's $r = .50$, $p < .001$; $r = .54$, $p < .001$, respectively). Therefore, we repeated our cortical thickness analyses after controlling for attention problem and ADHD scale CBCL scores, to ensure that these variables were not driving our results.

As three children from our overall sample had externalizing *t* scores within the clinical range when the CBCL was repeated on longitudinal follow-up and two additional children met criteria for an externalizing disorder during the study period, we repeated our analyses after excluding these children to confirm that these five subjects were not driving our overall results.

Results

Demographics

Descriptive statistics of the analyzed sample are presented in Table 1. After strict quality control of MRI data and exclusion of subjects without scores for the CBCL for children aged 6 to 18 years, our sample numbered 297 participants (164 female subjects) with 517 MRI scans and CBCL scores (age range = 6–18 years, mean = 12.1 ± 3.1). Raw CBCL externalizing scores ranged between 0 and 24 (*t* score = 32–69) (mean = 3.13 ± 3.4). Successful measurement of amygdala volume was completed in the same overall sample, but 13 subjects were excluded from network analyses due to a poor segmentation result ($n = 284$ participants, 492 MRI brain scans). Of note, a small number of participants from our sample (30 of 297 participants) met criteria for a nonexclusionary Axis I psychiatric disorder upon study enrollment or Axis I disorder upon longitudinal follow-up (Table 1).

Analysis 1. Regional Cortical Thickness and Externalizing Behavior

Negative linear associations between CBCL externalizing score and cortical thickness were found in: 1) left posteromedial OFC ($p = .04$, RFT-corrected, cluster level); 2) right retrosplenial cingulate ($p = .002$, RFT-corrected, cluster level); and 3) right medial temporal cortex ($p = .006$, RFT-corrected, cluster level) (Figure 1; Figure S1 and Table S2 in Supplement 1). Negative associations between externalizing score and 1) left posteromedial OFC, and 2) right retrosplenial cingulate cortex remained after controlling for attention problem and ADHD scale CBCL scores in our model ($p = .005$, uncorrected). No significant quadratic associations were found. Graphs plotting cortical thickness against externalizing behaviors illustrated that lower cortical thickness within the aforementioned regions was associated with higher CBCL externalizing scores, and vice versa. Exploratory analysis for age by externalizing score did not predict cortical thickness. A significant sex by externalizing score interaction effect on thickness in left parahippocampal cortex was found ($p = .0005$, RFT-corrected, cluster level) and driven by

Table 1. Descriptive Statistics

Age in Years	12.1 ± 3.1 (6–18)			
Gender	Male subjects: n = 133, total scans = 222	Female subjects: n=164, total scans = 295		
Raw CBCL Externalizing Score	3.13 ± 3.4 (0–24)			
Full Scale IQ	111.3 ± 12.5 (78–160)			
Handedness	Right = 266 (90%) Left = 31 (10%)			
Sample Statistics by Gender	**Male Subjects**	**Female Subjects**	***t***	***p***
Age in Years	12 ± 3 (6–18)	12.1 ± 3.2 (6–18)	$t_{1,295}$ = .76	.69
Raw CBCL Externalizing Score	3.29 ± 3.4 (0–14)	3 ± 3.5 (0–24)	$t_{1,295}$ = .63	.53
Full Scale IQ	112.82 ± 13.5 (78–160)	110.89 ± 11.6 (79–135)	$t_{1,295}$ = 1.87	.06
Handedness	Right = 116 (87%) Left = 17 (13%)	Right = 150 (91%) Left = 14 (9%)		
Axis I Disorders				
Nonexclusionary, present upon study enrollment	Specific phobia (n = 7) Separation anxiety (n = 1) Social phobia (n = 1) Enuresis (n = 3)	Specific phobia (n = 9) Separation anxiety (n = 1) Nicotine dependence (n = 1) ODD (n = 1)		
New-onset disorders, present on longitudinal follow-up	Conduct disorder (n = 1) Alcohol dependence (n = 2)	Alcohol dependence (n = 1)		

Participants: n = 297; 127 participants had data from one time point, 114 subjects had data from two time points, 54 subjects had data from three time points. All descriptive statistics are represented as means ± standard deviations; ranges for each item are presented in brackets.

CBCL, Child Behavior Checklist; IQ, intelligence quotient; ODD, oppositional defiant disorder.

gender differences in the slope of association between regional thickness and externalizing score ($t_{1,214}$ = 4.73, $p < .0001$; male subjects: r = .18, p = .007; female subjects: r = −.28, $p < .001$) (Figure S2 in Supplement 1). Adding attention problem and ADHD scale CBCL scores as control variables did not change these results.

Analysis 2. Amygdala Volumes and Externalizing Behavior

Amygdala volumes were not significantly associated with externalizing scores (left amygdala: $F_{1,204}$ = .32, p = .75; right amygdala: $F_{1,204}$ = −.4, p = .69). Interaction effects for age or sex by externalizing score on amygdala volume were not significant ($p > .05$).

Analysis 3. Cortico-Amygdalar Networks and Externalizing Behavior

Using our dimensional data, a significant interaction effect for left amygdala volume and externalizing score on thickness in left OFC (p = .003, RFT-corrected, cluster level) was found (Figure 2; Table S2 in Supplement 1). A trend toward a significant interaction effect between right amygdala volume and externalizing score on left OFC thickness was also identified (p = .005, uncorrected). No significant triple interaction effects for age or sex by externalizing score by left or right amygdala volume were found. Adding attention problem and ADHD scale CBCL scores as control variables did not change these results. On our planned follow-up categorical analysis, an externalizing group by left amygdala interaction effect on left OFC thickness was found (p = .0007, RFT-corrected, cluster level). A mixed-effects amygdala by group interaction model confirmed the presence of significant differences in the slope of association between amygdala volume and OFC thickness in low versus high externalizing groups ($F_{1,202}$ = 3.02, p = .003). A positive association between amygdala volume and OFC thickness was present in our low externalizing group, whereas these regions were not associated in our high externalizing group (Figure 2).

Excluding the 5 subjects (of 297 participants) with externalizing scores within the clinical range or DISC-measured externalizing disorder during the study period did not change the results reported above (i.e., for Analysis 1, 2, or 3).

Discussion

In the present study, we examined cortical thickness and cortico-amygdalar network correlates of externalizing behavior in a longitudinal sample of 297 healthy children, with 517 MRI brain scans and behavioral scores, from the NIH MRI Study of Normal Brain Development. To our knowledge, this is the first study to examine structural relationships between regional cortical thickness, amygdalae, and externalizing behaviors in children. We first found that thicknesses within left OFC, right retrosplenial cingulate, and medial temporal cortex were negatively correlated with externalizing behaviors. Amygdala volume was not correlated with externalizing behavior. However, an OFC-amygdala circuit was associated with externalizing behavior. Follow-up analyses indicated significant differences in relationships between amygdala volume and OFC thickness in children with lower versus higher externalizing behaviors. Children with lower externalizing behaviors exhibited positive correlations between amygdala volume and left OFC thickness, whereas this association was not present in children expressing higher rates of externalizing behaviors. When taken together, our findings: 1) identify key nodes in frontal, cingulate, and medial temporal cortex associated with externalizing behaviors in healthy children; and 2) identify a brain circuit (OFC-amygdala) that influences externalizing behaviors in children who have no identified psychopathology.

The OFC, retrosplenial cingulate, and medial temporal cortex are brain regions important for decision making and behavioral regulation (6,40,41). The medial prefrontal cortex (including OFC) is uniquely interconnected and linked extensively to cortical and subcortical structures (41). This cortical region is proposed to act as a hub area that helps to combine information to generate a

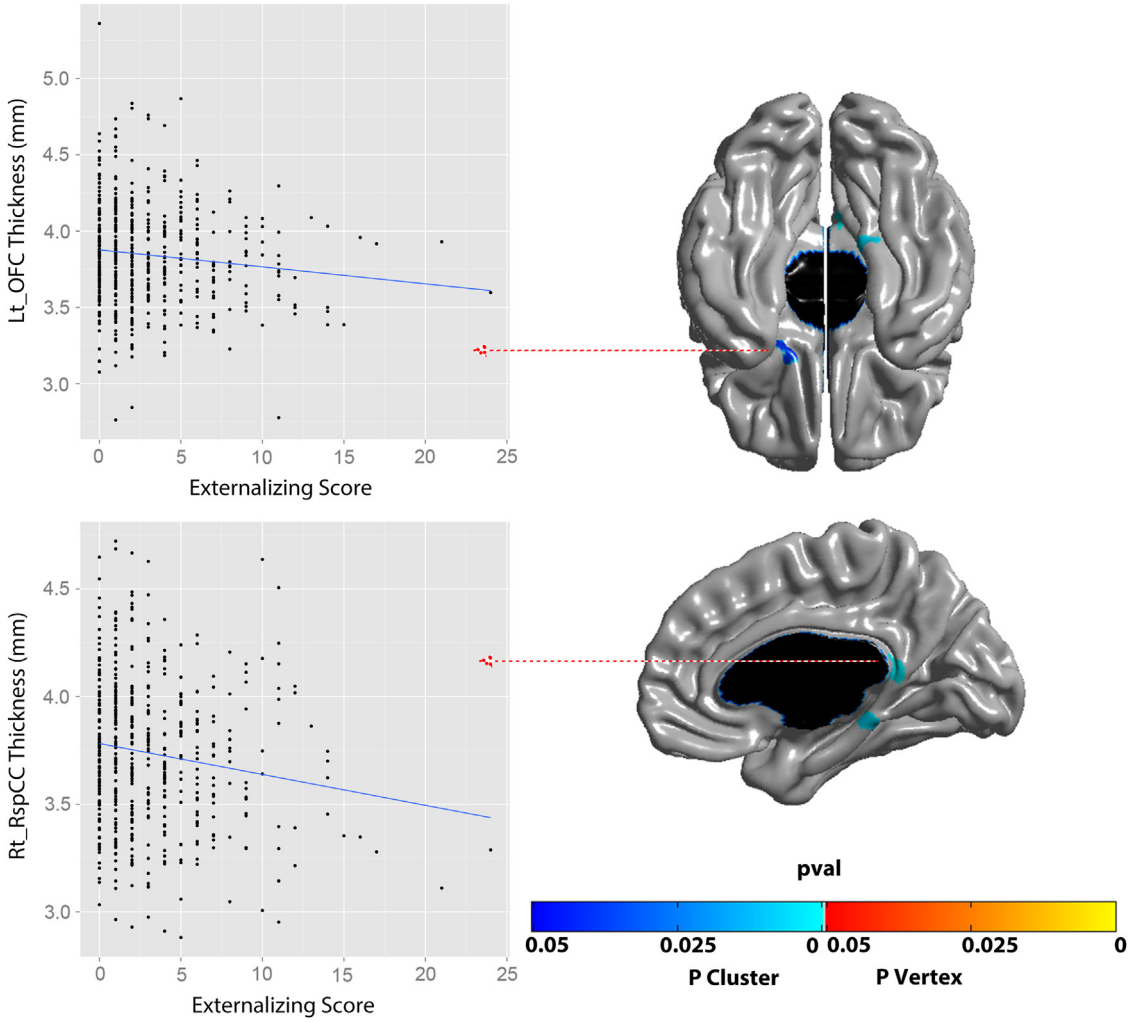

Figure 1. Negative associations between cortical thickness and externalizing scores. Right side of figure illustrates negative correlations found between raw Child Behavior Checklist externalizing scores and whole-brain cortical thickness projected onto brain templates. Random field theory was used to correct for multiple comparisons (figure is shown at $p \leq .05$, random field theory corrected). Blue areas are significant at the cluster level and red color corresponds to areas significant at the vertex level (none significant). Significant clusters were found within the left posteromedial orbitofrontal cortex, right retrosplenial cingulate cortex (Rt_RspCC), and medial temporal cortex. Linear regressions illustrating relationships between regional cortical thickness in left orbitofrontal cortex (Lt_OFC) (top) and Rt_RspCC (bottom) and externalizing scores are shown on left side of figure. Longitudinal data for all $n = 297$ participants are shown (i.e., $n = 517$ magnetic resonance imaging scans and Child Behavior Checklist scores). P, *p* value; pval, *p* value.

gestalt representation of a given situation and determine the most adaptive and appropriate response (41). Damage to the OFC often leads to externalizing behavior (42), with pronounced impairments seen when injury occurs during childhood (6). The retrosplenial cortex (part of the posterior cingulate gyrus) is involved in sensory monitoring of the internal and external environment (43). Within the default mode network, a functionally defined network engaged when one is left to think undisturbed, the retrosplenial cingulate cortex typically activates in response to personally significant situational information (40). Of interest, cortical regions that were correlated with externalizing behaviors in the present study overlap largely with a medial temporal subsystem of the default mode network, including ventromedial prefrontal, retrosplenial cingulate, and medial temporal cortex, which activates in response to tasks involving simulation of one's self in the future (40). Functions associated with OFC, retrosplenial cingulate, and medial temporal cortex indicate that these structures may regulate externalizing behaviors through their role in situational processing, accurate prediction of future events (and personal consequences), and appropriate response selection.

Our results are in line with previous work in children with psychiatric disorders, suggesting that thinner cortex within key cortical nodes may increase propensity for externalizing behaviors. Two previous cross-sectional studies have found thinner cortex in children and adolescents with ODD and conduct disorder, with effects in medial frontal and retrosplenial/posterior cingulate cortex (8,44). Longitudinal mapping of cortical thickness has also indicated that decreased medial prefrontal cortex thickness in childhood ADHD predicts poorer clinical outcome in adolescence (45). Of note, increased gray matter volume and concentration within OFC has also been shown in boys with elevated levels of callous unemotional conduct problems compared with control subjects (46). As cortical volume is the product of two different dimensions of the cortical sheet, surface area and cortical thickness (47), differences in the direction of these findings may be due to the use of a different structural index (i.e., volume

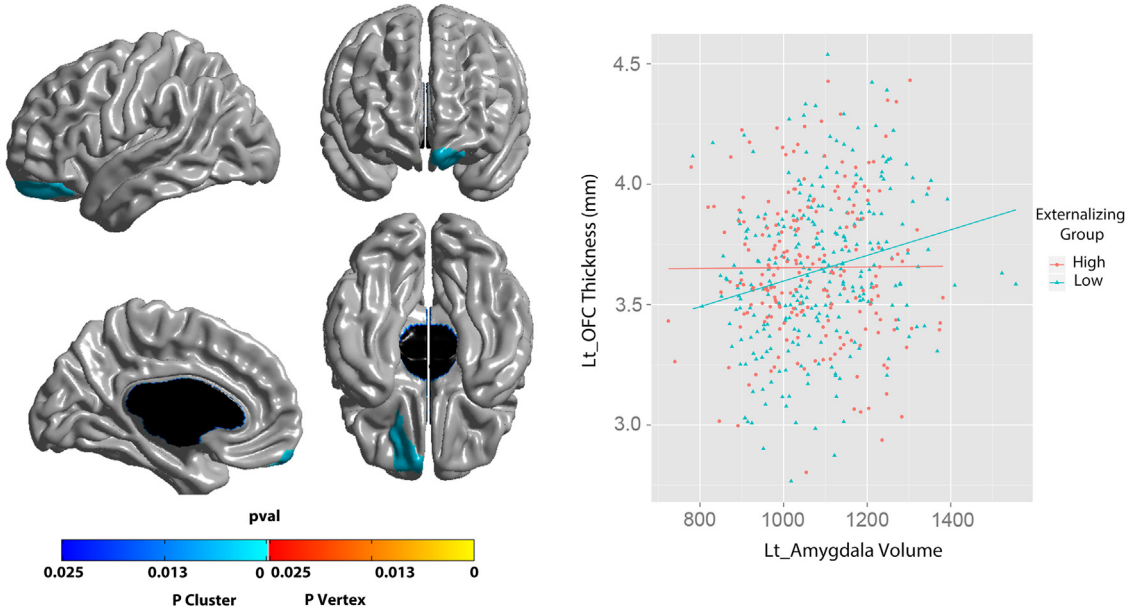

Figure 2. Cortico-amygdalar network correlates of externalizing behavior. Left side of figure illustrates the left orbitofrontal cortex (Lt_OFC) region where relationships between cortical thickness and left amygdala (Lt_Amygdala) volume differed according to raw Child Behavior Checklist (CBCL) externalizing scores. Random field theory was used to correct for multiple comparisons of cortical thickness values across the cortex. In addition, a Bonferroni correction was applied to correct for comparisons of left and right amygdala volume. Figure is shown at $p \leq .025$, random field theory-corrected. Blue areas are significant at the cluster level and red color corresponds to areas significant at the vertex level (none significant). Scatterplot on right side of figure illustrates differences in the relationship between Lt_OFC thickness values and Lt_Amygdala volume in low (raw CBCL externalizing score = 0–2, $n = 280$, blue triangles) versus high externalizing groups (raw CBCL externalizing score ≥ 3, $n = 212$, pink circles). Longitudinal data for all $n = 297$ participants are shown (i.e., $n = 517$ magnetic resonance imaging scans and CBCL scores). Significant differences in slopes for correlations between amygdala volume and orbitofrontal cortex thickness in low versus high externalizing groups were found ($F_{1,202} = 3.02$, $p = .003$). P, p value; pval, p value.

vs. thickness) or perhaps signal alterations that are specific to boys with callous unemotional traits, thought to be an antecedent to psychopathy (46). Previous work has speculated that genetic factors, white matter development, and use-dependent selective pruning of synapses influence thickness in the developing cortex (48). Further research is now needed to clarify the microstructural properties and genetic and environmental factors underlying regional cortical thickness variations that are associated with externalizing behaviors among children. In addition to negative associations found between regional cortical thickness and externalizing scores in our overall sample, exploratory analyses uncovered differences in patterns of association between left parahippocampal cortex thickness and externalizing scores in boys versus girls in our sample. Although the significance of these results is unclear, it is known that externalizing scores are increased in boys compared with girls in clinical and healthy samples (1). In girls within our sample, externalizing scores explained over twice the variance in left parahippocampal cortex thickness compared with boys (8% vs. 3%). Therefore, structural variation within this cortical region may be a susceptibility factor for externalizing behavior that is of particular importance in girls. Our results suggest that further examination of the effects of gender on relationships between cortical structure and externalizing behavior is needed to better characterize how brain differences are associated with gender disparity in this behavioral realm.

Using a network-based structural covariance approach, we found that high versus low externalizing behavior predicted structural correlations between amygdala volume and left OFC thickness. While OFC thickness and amygdala volume were positively correlated in children expressing few externalizing behaviors, structure within these regions was not correlated in children expressing higher rates of externalizing behaviors. To our knowledge, this is the first direct demonstration of the importance of structural relationships between cortico-amygdalar gray matter networks and externalizing behavior. Alterations in structural white matter connections and altered functional connectivity has been found between OFC and amygdala, in children and adolescents with ODD or conduct disorder versus control subjects (12,13) and in adults with high rates of antisocial behaviors (49). Therefore, our results, obtained from a healthy sample, should add confidence to previously discovered cortico-amygdalar findings reported in externalizing disorder samples, where medication, substance use, and other confounds that can affect brain structure and function are often present.

The strong concordance of our findings in a nonclinical sample with findings in clinical samples supports the dimensionality of relationships between fronto-amygdala circuit properties and externalizing behavior. Our structural network findings mirror the results of a recent fMRI study, which found that youths with externalizing disorders lack the positive functional connectivity found between OFC and amygdala in control subjects during a moral judgment task (13). In this study, reduced OFC-amygdala functional coupling in externalizing disorder youths was interpreted as a marker of impaired OFC-mediated control over amygdala activity. It has been demonstrated that structural covariance between brain regions parallels functionally defined networks (50–52). Therefore, we speculate that reduced structural coupling between OFC and amygdala, as found here in healthy children with higher externalizing behaviors, may contribute to risk for externalizing behaviors along a continuum. Prior work has shown that OFC thickness is correlated with fMRI-measured suppression of amygdala activity during emotion regulation in healthy adults, indicating that OFC structure relates to amygdala function (53). Positive structural associations within this circuitry

may therefore work to inhibit externalizing behaviors through optimal top-down regulatory control over amygdala output. In contrast, the absence of structural associations between OFC and amygdala volume may be a susceptibility factor contributing to increased vulnerability to externalizing behavior expression via diminished OFC over amygdala regulatory control or unchecked amygdala activation.

Some limitations of the present findings require consideration. First, although 5 of 297 participants within our sample had either clinically significant CBCL-derived externalizing symptoms or DISC-measured externalizing disorders, overall our sample did not include children with externalizing disorders, and therefore, we cannot be certain that our results generalize to clinical populations. However, the clear parallel findings in clinical populations, coupled with the fact that our sample is absent of medication and substance use confounds that are frequently present in clinical populations with externalizing behaviors, should provide confidence in our results. Further, our data were acquired across multiple sites and on different MRI scanners, which could potentially introduce between-site variability with respect to brain structure measurements. Reliability between sites was therefore monitored carefully using phantoms throughout this study's data acquisition phase. In addition, scanner was used as a covariate in our analyses.

Conclusions

In conclusion, our results indicate that reduced thickness of OFC, retrosplenial cingulate, and medial temporal cortex may increase susceptibility to externalizing behavior expression in children. Using a network-based approach, our data also show that the absence of positive structural relationships between amygdala and OFC may confer susceptibility for externalizing behaviors. Conversely, positive associations between amygdala and OFC may protect against susceptibility for externalizing behaviors. The identification of underlying brain circuits important for externalizing behaviors is an important step toward targeted treatment, since recent studies indicate that network properties may change with therapeutic intervention (54). Overall, our results highlight cortical thickness and cortico-amygdalar network properties that may influence externalizing behavior regulation and provide novel insight into circuit variation that may confer risk for externalizing behaviors, along a continuum, and across healthy and clinical samples. Future research is needed to determine whether fronto-amygdala circuitry can be used as a treatment biomarker for behavioral and pharmacologic interventions aimed at improving externalizing behaviors.

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