Functional Connectivity of the Amygdala in Early-Childhood-Onset Depression

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Objective: Adult major depressive disorder (MDD) is associated with reduced cortico-limbic functional connectivity thought to indicate decreased top-down control of emotion. However, it is unclear whether such connectivity alterations are also present in early-childhood–onset MDD. **Method:** A total of 51 children 7 through 11 years of age who had been prospectively studied since preschool age, completed resting state functional magnetic resonance imaging (fMRI) and were assigned to one of four groups: 1) C-MDD (N = 13), those children with a personal history of early-childhood–onset MDD; 2) M-MDD (N = 11), those with a maternal history of affective disorders; 3) CM-MDD (N = 13), those with both maternal and early-childhood–onset MDD; or 4) CON (N = 14), those without either a personal or maternal history of MDD. We used seed-based resting state functional connectivity (rsfcMRI) analysis in an independent sample of adults to identify networks showing both positive (e.g., limbic regions) and negative (e.g., dorsal frontal/parietal regions) connectivity with the amygdala. These regions were then used in region-of-interest–based analyses of our child sample. **Results:** We found a significant interaction between maternal affective disorder history and the child's MDD history for both positive and negative rsfcMRI networks. Specifically, when compared with CON, we found reduced connectivity between the amygdala and the "negative network" in children with C-MDD, M-MDD, and CM-MDD. Children with either C-MDD or a maternal history of MDD (but not CM-MDD) displayed reduced connectivity between the amygdala and the "positive network." **Conclusions:** Our finding of an attenuated relationship between the amygdala, a region affected in MDD and involved in emotion processing, and cognitive control regions is consistent with a hypothesis of altered regulation of emotional processing in C-MDD, suggesting developmental continuity of this alteration into early childhood. J. Am. Acad. Child Adolesc. Psychiatry, 2011;50(10):1027–1041. **Key Words:** functional connectivity, depression, amygdala, fMRI, childhood-onset

There is growing empirical evidence suggesting that clincially signficant episodes of major depressive disorder (MDD) can be identified and reliably diagnosed in preschool and early school aged children when age-adjusted *DSM* criteria are implemented.[1-5] However, little is known about the neural correlates of the illness in this age group. Understanding the neural correlates in the earliest known forms of MDD will provide clues necessary to characterize the neurodevelopmental pathophysiology of this disorder. The adult and adolescent depression literatures point to behavioral and neural problems with emotion regulation, or the ability to effectively and adaptively control negative thoughts, feelings, and moods.[2,6,7] Furthermore, functional magnetic resonance imaging (fMRI) studies highlight the importance of a network of regions for emotion regulation, including the amygdala and various regions of the prefrontal cortex,[8-10] anterior cingulate, dorsolateral prefrontal cortex, ventral lateral prefrontal cortex, and the medial prefrontal cortex.[6,11-15] Based on the evidence that problems with emotion regulation are an important component of MDD and that emotion regulation involves a host of regions affected in adult and adolescent MDD, we used a resting state functional connectivity ap-

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proach to investigate amygdala connectivity with the prefrontal cortex and other cortical regions, as well as with other limbic regions, in children 7 through 11 years of age who had been propsectively studied since the preschool period and were known to have early-onset MDD and/or increased risk for MDD on the basis of family history of the disorder.[6,11-15]

MDD has been well validated and widely recognized in children for decades. Preschool-onset MDD has been shown more recently to share core clinical characteristics with adolescent and adult-onset MDD, as well as significant homotypic continuity with later childhood forms of MDD, suggesting that it is an early-onset form of the well-known lifespan disorder.[5,16] Problems with emotion regulation are well established in both childhood and adult MDD; both age groups have difficulty generating and maintaining/recognizing appropriate positive emotions and also experience an inordinate amount of intense negative emotions including sadness, guilt, and feelings of worthlessness.[17] The importance of understanding and successfully treating MDD in childhood is underscored by findings that children and adolescents with greater MDD episode duration show greater risk of relapse.[18-21] It is important to understand the specific neural systems affected in pediatric MDD, not only because childhood onset of the disease is linked to a poorer lifetime prognosis, but also because these children are affected during critical years for cognitive, social, and neural development and because currently effective treatment options are limited.[22]

The Amygdala and Emotion Regulation in MDD

Task-based fMRI studies investigating emotion processing in depressed samples report increased responses to negative emotional cues (e.g., faces, pictures, words) in limbic regions, including the amygdala, along with altered activity in cognitive control regions during emotional distraction and regulation.[12,23-37] This pattern of hyperactivity of limbic regions, including the amygdala, while viewing emotional faces has received mixed support in the adolescent MDD literature.[38-40] Interestingly, adolescents with MDD also display reduced activation in dorsal prefrontal and cingulate cortex during cognitive control tasks.[41] There is also evidence for altered pregenual cingulate activity during an emotional stroop task and increased amygdala response to fearful face stimuli in school-aged children at high risk for MDD.[42] Many of these regions affected in MDD are implicated in emotion regulation processes in healthy populations.

Specifically, regions such as the dorsolateral prefrontal cortex and the medial prefrontal cortex are thought to modulate activity of regions involved in emotionality, such as the amygdala.[8] During successful emotion regulation, both dorsal and medial prefrontal regions display increased activity, along with a decrease in amygdala activity. Moreover, stronger connectivity between these regions is associated with a greater reduction in negative affect after attempts to regulate emotion.[43] This evidence, along with the amygdala's central role in assigning affective salience to stimuli and involvement in emotional memory, attention, and fear conditioning, suggests that it may be a critical node in the pathway through which regions involved in control influence the activation of emotion regulatory processes.[44-46]

Investigating the functional relationship (i.e., functional connectivity) between the amygdala and other emotion regulation regions may help to elucidate the dynamics of regulation and inform our understanding of brain system alterations that contribute to maladaptive emotion regulation processees associated with MDD. Work in healthy adults has shown that the timing of, and relationship between, activity in the amygdala and regions of the prefrontal cortex (dorsolateral, dorsomedial, and orbital) may vary, depending on the efficacy of emotion regulation and type of regulation strategy used.[9,43,47] Thus, even in the absence of the use of an explicit emotion regulation task in the scanner, one can make inferences about the regions involved in emotion regulation using resting state functional connectivity, as explained below.

Resting State Functional Connectivity of the Amygdala

As mentioned above, research has found the amygdala to be intricately linked with both cortical and subcortical regions during tasks involving preattentive and purposeful emotion regulation and recognition. Interestingly, a similar pattern of relationships has also been found using resting state functional connectivity MRI (rsfcMRI). Data collected during resting state functional connectivity measures spontaneous low-frequency blood oxygen level–dependent

(BOLD) activity, whereas an individual is "at rest" (i.e., lying quietly with eyes open or closed). This technique has been used to demonstrate "functional connectivity" (i.e., similar patterns of fluctuation in the BOLD signal) among brain regions.[48] Importantly, research using resting state functional connectivity has demonstrated that groups of brain regions that often activate (or deactivate) at the same time in task settings also exhibit functional connectivity at rest, suggesting a history of coactivation that can be used to examine functional relationships within the brain. A growing body of literature using this technique has identified functional brain systems related to basic sensory functions such as vision and somatosensory processes as well as networks of regions related to task control, attention, and default mode activity. As such, resting state functional connectivity is a useful tool for examining the participation of a given brain region within a larger neural system, as well as identifying the potential influence of early occurring psychopathology on these functional relationships.

Stein et al. used path analysis to examine a network of regions displaying functional connectivity with the amygdala, including cortical structures such as the cingulate, prefrontal, and insular cortices along with the hippocampus and parahippocampal gyrus.[49] Recently, Roy et al. investigated amygdala connectivity using seed-based resting state functional connectivity techniques in healthy adults.[50] Many regions involved in the generation, regulation, and/or memory of emotions demonstrated negative or positive resting state functional connectivity relationships with the amygdala. More specifically, regions typically involved in control processes (frontal/parietal regions) displayed a negative functional relationship with the amygdala, which was interpreted as a potential method of top-down regulation of amygdala activity by these control regions. Conversely, limbic regions such as the hippocampus displayed a positive functional relationship with the amygdala.

Amygdala Functional Connectivity in MDD
Studies investigating functional connectivity of the amygdala in adult MDD have revealed some important differences between patient and control populations. Chen et al. found reduced connectivity among individuals with MDD between the amygdala and both control and limbic re-

gions that reversed after selective serotonin reuptake inhibitor (SSRI) treatment.[51] In a series of studies, Anand et al. found decreased cortico-limbic connectivity in individuals with MDD both at rest and while viewing emotional pictures.[52-54] Further studies indicated that treatment with an SSRI served to increase cortico-limbic connectivity. There is also evidence that amygdala–prefrontal cortex connectivity varies along with monoamine oxidase A genotype, such that depressed individuals and persons with the monoamine oxidase A-H variant show decreased functional connectivity.[55] Matthews et al. found reduced amygdala to supragenual cingulate connectivity in MDD patients, with greater reductions in connectivity related to greater symptom severity.[56] Lui et al. found reduced resting state functional connectivity within a network of regions involved in emotion regulation; interestingly, whereas the refractory MDD group showed this decrease compared with controls, a nonrefractory MDD group showed even further reduction in thalamo-cortical functional connectivity.[57] Cullen et al. found reduced fractional anisotropy between the amygdala and right subgenual ACC in adolescents with MDD, using diffusion tensor imaging, indicating reduced white matter connectivity between these regions.[58] A recent study by Gaffrey et al. focused on functional connectivity of the subgenual cingulate, but not the amygdala, using a sub-sample of children with preschool-onset MDD from the same study, and found reduced connectivity between the subgenual cingulate and prefrontal control regions.[59] However, to date, no studies have examined amygdala resting state functional connectivity in children with early-childhood–onset MDD.

Amygdala Function in Children at Risk for MDD
Connections among regions involved in emotion regulation may also be altered in individuals at risk for developing MDD. Children with a parental history of MDD face a risk of developing MDD that is three times greater than that of same-age peers with no parental history of MDD.[60] Several behavioral and fMRI studies have been conducted with high-risk groups. These studies suggest that neural and behavioral differences related to emotion dysregulation (and similar to those found in adolescents and adults with MDD) are present in children who have never displayed MDD but are at high risk for

developing the disease.[61-63] However, to our knowledge, no studies have investigated differences in functional connectivity within a familial high-risk population. Studying resting state functional connectivity within emotion regulation networks in individuals at high risk and low risk for developing MDD, in addition to those with early-onset MDD, may inform our understanding of whether changes in the connectivity of these networks is a symptom of MDD or predates clinical onset of the disease.

Goals and Hypotheses of the Current Study

The present study used resting state functional connectivity to investigate amygdala connectivity in children with a past episode of C-MDD and those at risk for later MDD. We focused on amygdala connectivity because of its central role in affective processing, along with past work in adults with MDD suggesting disrupted amygdala connectivity.[46,51] Given the evidence for altered connectivity patterns among adults with MDD, we hypothesized that children who had an episode of MDD during early childhood and those at risk for MDD would also show reduced connectivity between the amygdala and prefrontal and cingulate regions thought to be important for the regulation of emotion. Our predictions were less clear in regard to positive connectivity between the amygdala and other limbic regions involved in the expression of emotional experience or the retrieval of emotional information, as the existing literature does not point to a clear hypothesis. As such, our investigation of differences in connectivity of the "positive network" among the three MDD groups was exploratory.

METHOD

Participants

Beginning in 2002, caregivers with children 3 through 6 years of age were recruited to participate in a longitiudinal study of preschool-onset depression. Prospective data continued to be collected from the original sample at annual assessment waves conducted in the Early Emotional Development Program at Washington University School of Medicine in St. Louis, MO; details of the recruitment process have been previously published.[5] Participants (7 through 11 years of age) in the current investigation included children with the following: 1) a history of MDD during early childhood (before age 8 years) (C-MDD; n = 13); 2) a maternal history of affective disorders

(M-MDD; n = 11); 3) both maternal and C-MDD (CM-MDD; n = 13); or 4) no C-MDD or maternal history (CON; n = 14). It is important to emphasize that placement in either C-MDD or CM-MDD group was based on past MDD diagnosis, and that although current depressive symptoms were included in subsequent analyses, diagnostic data from time of scan were not avaliable for these analyses. In addition, children were placed in the CON group if they did not have a personal MDD history or maternal history of affective disorders, regardless of other diagnoses, although any comorbidies were included as covariates in the analyses. The criteria for excluding participants based on movement and the number of participants excluded are discussed under fcMRI data processing. Table 1 lists relevant demographic and clinical characteristics of the participants. The C-MDD group had significantly lower family income than all other groups ($p <$ .05), but no other groups differed significantly. The C-MDD group had significantly lower maternal education ($p <$ .05) than the M-MDD and CM-MDD groups, but no other groups differed significantly. Because education level and income are highly correlated ($p <$ .0001), only maternal education level was used as a covariate in further analyses. The study was approved by the Washington University institutional review board. All parents or guardians gave written informed consent, and all participants gave written or oral assent after a description of the risks and benefits of study participation.

Diagnosis and Individual Differences

Trained staff members conducted up to three annual in-person interviews with the participants and parents/guardians over the course of 4 to 6 years. These interviews included the Preschool-Age Psychiatric Assessment (PAPA), a semi-structured developmentally appropriate parent assessment of psychiatric symptoms with established reliability.[64] PAPA interviews were audiotaped and reviewed for reliability as previously reported.[5] The PAPA uses a computerized *DSM-IV* algorithm to derive MDD and other Axis I diagnostic determinations. However the 2-week duration of symptoms requirement for the diagnosis of MDD was omitted based on empirical data suggesting that it may not be an appropriate duration threshold for younger children.[4] Using information from the PAPA, children were classified as having a C-MDD history if the child met all symptom criteria for MDD or had anhedonia along with three other symptoms at one or more of the annual assessments. Maternal affective disorder history was determined using the Family Interview for Genetic Studies (FIGS), a widely used and reliable measure of family history of psychiatric disorder administered by trained research assistants.[65] Participants were considered part of the maternal history group if their primary caregiver reported the child's biological mother as ever having

TABLE 1 Demographic and Clinical Characteristics of the Participant Groups

Characteristic	Child Only MDD (C-MDD) n = 13	Maternal Only MDD (M-MDD) n = 11	Child and Maternal MDD (CM-MDD) n = 13	Neither Child nor Maternal MDD (CON) n = 14
Gender (% male)	38.5	45.5	61.5	42.9
Age (y)*				
Mean	8.9	9.1	9.5	8.4
SD	.62	1.0	1.1	0.9
Range	8–10	8–11	8–11	7–10
Ethnicity (% white)*	38.4	72.7	76.9	64.3
Psychiatric medication at diagnostic time point before scan (%)[a]	30.8	18.2	23.1	14.3
Family income level*[b]	1.85	2.8	3.25	3.25
Maternal education level*[c]	4.79	5.73	6.69	5.93
Comorbidities (%)**				
Mother: other internalizing diagnosis	0	27.3	23.1	0
Mother: externalizing disorder diagnosis	23.1	18.2	0	7.1
Child: other internalizing disorder diagnosis	53.8	9.1	69.2	7.1
Child: externalizing disorder diagnoses	61.5	36.4	76.9	14.3
Child Depression Inventory (child form) total T score (parent form)	40.9 (2.558)	44.8 (6.374)	43.25 (6.369)	40.9 (2.558)
Child Depression Inventory total T score (parent form)	44.2 (9.175)	51.6 (7.351)	50.17 (7.107)	44.2 (9.175)
Time point 1 depression sum score**	2.50 (2.41)	2.64 (2.618)	6.31 (4.404)	2.5 (2.410)
Mean depression sum score** (time points 1, 3, and 5)	1.60 (1.68)	2.67 (.9428)	7.128 (2.577)	1.595 (1.680)

Note: MDD = major depressive disorder.

[a]Results were essentially identical if children on medications were excluded from the analyses, or if medication status was used as a covariate.

[b]Family income per year was obtained at the first diagnostic wave using the following categories: 1 = $0–20,000, 2 = $20,001–40,000, 3 = $40,001–60,000, and 4 = $60,001+.

[c]Maternal education level was obtained using the following categories: 1 = some grade school; 2 = completed grade school, 3 = some high school, 4 = completed high school, 5 = some college or completed a 2-year degree, 6 = completed a 4-year college degree, 7 = some school beyond college, and 8 = completed a professional or graduate degree.

*p < .05, **p < .01.

had MDD or bipolar disorder. Two mothers in the M-MDD group were diagnosed with bipolar disorder. In the control group, one reporter of maternal MDD/bipolar history was the child's biological father and one was an adoptive parent, and in the C-MDD group one reporter was the child's guardian but not a biological or adoptive parent or a grandparent. All other reports were from the biological mothers about themselves. Participants were excluded from the following analyses if maternal affective disorder history was missing.

Along with the MDD module, all other relevant diagnostic modules of the PAPA were administered to determine whether the following disorders were present at any of the three study waves: separation anxiety disorder, post-traumatic stress disorder, generalized anxiety disorder, attention-deficit/hyperactivity disorder, conduct disorder, specific phobia, and oppositional defiant disorder. The CON and M-MDD groups showed significantly fewer internalizing diagnoses

than the C-MDD and CM-MDD groups. The CON group showed significantly fewer externalizing diagnoses than the C-MDD and CM-MDD groups, whereas the M-MDD group showed significantly fewer externalizing diagnoses than the CM-MDD group only. To control for possible influences of early childhood comorbidities, we included prior diagnosis of other internalizing and/or externalizing disorders as covariates in subsequent analyses.

At each annual diagnostic assessment, depression sum scores or depression severity scores (the total number of MDD symptoms endorsed in the PAPA) were calculated and used as a proxy for symptom intensity severity.[66] To evaluate current depressive symptoms, the Child Depression Inventory (CDI) was administered on the same day as children's fMRI scanning. All children were more than 6 years of age, the lower age limit of the CDI, at the time of scan.[67] Children and caregivers completed the child and parent versions, respectively. Scores from seven children

were not available (four CON, one C-MDD, one M-MDD, and one CM-MDD). Total CDI scores were computed for both informants and converted to T scores. The Childhood Emotional Management Scale (CEMS) was used to measure children's ability to cope with and regulate experiences of sadness.[68] The CEMS was administered at the annual assessment before the scanning session. Diagnostic groups did not significantly differ in CEMS scores or in parent/child CDI scores (all $p > .05$). Children's sadness dysregulation and coping scores on the CEMS, MDD sum scores from T1, and the mean of MDD sum scores across all three annual diagnostic waves (T1, T2, and T3) were used to investigate individual differences in connectivity associated with MDD severity and emotion regulation ability.

fMRI Scanning

Two resting state fMRI scans, each including 164 frames (each lasting ~6.8 minutes), were acquired using a 3T Tim TRIO Scanner at Washington University School of Medicine. Subjects were instructed to remain awake and rest with their eyes closed during the resting state scan. Data were acquired using an asymmetric spin-echo, echo-planar sequence, which was maximally sensitive to blood oxygenation level–dependent (BOLD) contrast (T2*) (repetition time [TR] = 2500 ms, echo time [TE] = 27 ms, field of view [FOV] = 256 mm, flip=90°, voxel size = 4 × 4 × 4 mm, slices = 36). In addition, a T1 structural image was acquired for alignment purposes using a sagittal MP-RAGE three-dimensional (3D) sequence (TR = 2400 ms, TE = 3.16 ms, flip = 8°; voxel size = 1 × 1 × 1 mm). To facilitate registration of the T1 and functional scans, we also acquired a T2 image in the same space as the functional scans (TE = 96 ms, TR = 5s, 189 × 256 acquisition matrix, 36 slices, voxel size = 1.0 × 1 × 3 mm).

fcMRI Data Preprocessing

Before processing, the signal-to-noise values (mean across time points within a run, divided by the standard deviation across time points, computed for each slice) were examined for each resting state scan for each child. We considered a scan to be of usable quality if the mean signal-to-noise value across slices was 150 or greater, as our prior studies have suggested that this cut off predicts a number of other quality assurance indices. Using this criterion, both scans from 25 children were excluded (CON, n = 11; PH-MDD, n = 3; M-MDD, n = 3; and CM-MDD, n = 8), one of the two scans from 11 children was excluded (CON, n = 3; PH-MDD, n = 2; M-MDD, n = 3; and CM-MDD, n = 3), and both scans from all remaining children were used, bringing our total number of subjects from 76 chidren scanned to 51 children for our analyses. We then compared signal-to-noise for the included children and scans across groups, and found no significant

differences ($F_{3,50} = 0.504$, $p = .681$). Data were then processed using the following steps before fcMRI analysis:

1. Compensation for slice-dependent time shifts
2. Removal of first five images from each run during which fMRI signal was allowed to reach steady state
3. Elimination of odd/even slice intensity differences due to interpolated acquisition
4. Realignment of data acquired in each subject within and across runs to compensate for rigid body motion[69]
5. Intensity normalization to a whole brain mode value of 1,000
6. Registration of the 3D structural volume (T1) to the atlas representative template in the Talairach coordinate system[70] using a 12-parameter affine transform and re-sampled to 1-mm cubic representation[69,71]
7. Co-registration of the 3D fMRI volume to the T2, and the T2 to the participant's structural image
8. Transformation of the fMRI to atlas space using a single affine 12-parameter transform (derived from the combination of steps 6 and 7) that included a re-sampling to a 3-mm cubic representation.

Finally, as described elsewhere,[72,73] to remove possible sources of spurious correlations, such as movement, scanner, and physiological noise, a number of additional preprocessing steps were performed using in-house software written in Matlab (Mathworks, Natick, MA). Briefly, the steps included the following: spatial smoothing using a 6-mm full-width half-maximum Gaussian filter; high-pass filtering at 0.009 Hz; and removal of several nuisance regressors, including six rigid-body motion correction parameters, ventricle, and whole-brain and deep white matter signal, as well as their first derivatives.[72,74,75]

Network Region Definition

We were interested in examining the relationship between the amygdala and both cortical and limbic regions. To do this, we identified networks of regions that showed both positive and negative functional correlations with both left and right amygdala using an independent data set of 24 healthy adults previously analyzed by our group.[73] To do so, we first created seed maps of regions showing functional connections with the right and left amygdala respectively. Specifically, the time series for all voxels within either a left or right amygdala ROI (anatomically defined for each adult individual using FreeSurfer segmentation)[76,77] were extracted and correlated with all other voxels in the brain. We estimated group-level statistical significance by converting individual correlation maps to Fisher Z maps and computing a voxelwise one-sample t test (comparing the Fisher Z values against zero across the group). We thresholded these

FIGURE 1 Negative and postive network regions of interest (ROIs). Note: Figure depicts ROIs comprising the (A) negative network (blue) and (B) positive network (red), along with the bilateral amygdala (yellow). Tables S1 and S2, available online, provide region coordinates and abbreviation expansions.
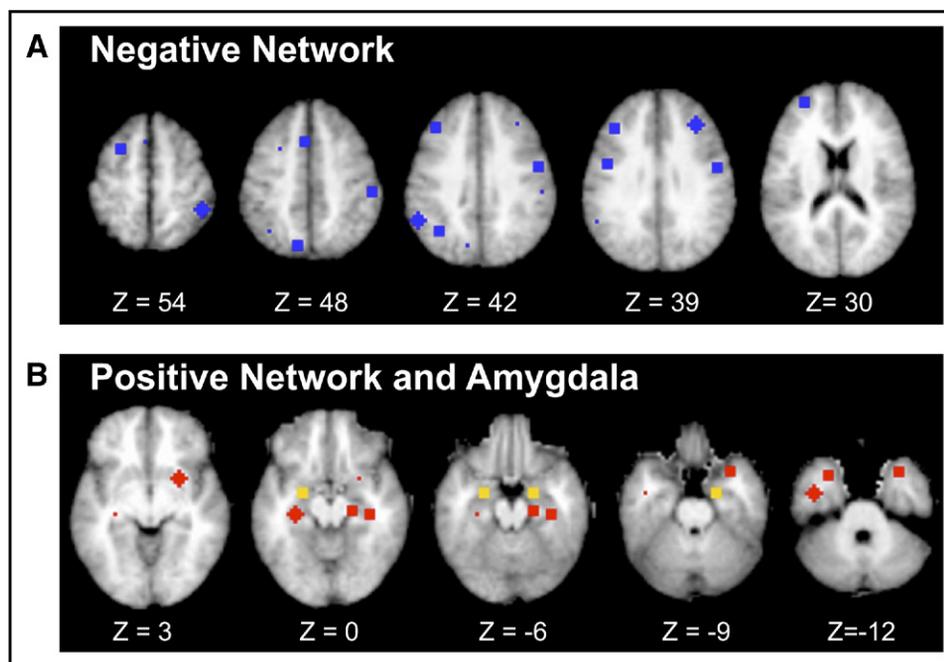
maps with a Z of 3 and 13 contiguous voxels to achieve a whole-brain false-positive rate of .05 for each of the two maps.[78] We then identified all voxels that passed threshold in both maps, and created two networks of regions that included regions either positively or negatively correlated with the bilateral amygdala, respectively along with group-level amygdala regions of interest. These two networks are shown in Figure 1, and the coordinates for the centroid of each region are shown in Tables S1 and S2 (available online). Overall, regions whose activity correlated positively with amygdala activity ("positive" network; Table S1, available online) tended to be more inferior "limbic" regions, whereas regions whose activity was negatively correlated with amygdala activity ("negative" network; Table S2, avaliable online) tended to be more dorsal frontal/parietal regions often associated with various forms of cognitive control.[79-82] Of note, the maps for right and left amygdala seed correlations were highly similar, and the results would not have been substantively different if we had identified separate positive and negative networks for the right and left amygdala and used those in subsequent analyses. A conjunction analysis was performed on two maps using either the left or right amygdala as seeds. This provided networks with the same regions for both right and left amygdala and allowed us to conduct symmetrical analyses with amygdala hemisphere as a factor and to increase the ease of presentation.

ROI–ROI Correlation Computation

In our child sample, we used the centroids of the regions, including the amygdala, listed in Tables S1 and S2 (available online), to create spherical ROIS 12 mm in diameter. These ROIs were then masked by each child's individual FreeSurfer Segmentation so as to include only voxels falling within gray matter. We then extracted the time series from each of these ROIs and computed pairwise correlations between each ROI and the left and right amygdala separately.

Data Analysis

To compare connectivity between the groups, we conducted a repeated-measures analysis of variance (ANOVA) for each network (positive and negative) with region (Tables S1 and S2, available online) and hemisphere (right or left amygdala) as within-subject factors, and maternal affective disorder status and child MDD status as between-subject factors. To control for possible confounds, gender, age, ethnicity (white, African American, or other), past diagnosis of any externalizing disorders (oppositional defiant disorder, conduct disorder, attention-deficit/hyperactivity disorder), past diagnosis of any other internalizing disorders (generalized anxiety disorder, seasonal affective disorder, post-traumatic stress disorder), medication status at scan (whether child was taking psychotropic medications during the diagnostic time

period before scan (Table S3, avaliable online), and parent-reported CDI score were included as covariates. No significant differences in results from those presented with the full sample were found when children on medications were excluded in follow-up analyses. Because of our interest in changes in connectivity between children with personal and maternal affective disorders compared with control children, and in the interest of brevity, we focused on main effects and interactions among these factors only.

RESULTS

Within-Network Connectivity

Negative Network. The ANOVA for the negative network did not reveal a significant main effect of either child MDD history ($F_{1,48} = 1.809$, $p = .185$) or maternal affective disorder history ($F_{1,48} = 1.631$, $p = .208$). However, there was a significant interaction between C-MDD history and maternal affective disorder history ($F_{1,48} = 4.805$, $p = .033$). This interaction remained significant after adding age, gender, ethnicity, maternal education level, past diagnoses of any internalizing or externalizing disorder, and parent CDI score as covariates ($F_{1,32} = 10.215$, $p = .003$). Post hoc analyses performed to identify the source of this interaction revealed decreased negative connectivity in the groups with personal (C-MDD; $p = .012$), maternal (M-MDD, $p = .02$), and both histories (CM-MDD; trend level, $p = .064$) when compared with controls (Figure 2a). In other words, all three groups with either or both a personal history of MDD or a maternal history of MDD showed reduced negative connectivity between the amygdala and regions such as dorsal prefrontal and parietal cortices compared with healthy children.

Positive Network. The ANOVA for the positive network did not reveal a significant main effect of either C-MDD history ($F_{1,48} = 0.575$, $p = .452$) or maternal affective disorder history ($F_{1,48} = 0.010$, $p = .922$). However, there was a significant interaction between C-MDD history and maternal affective disorder history ($F_{1,48} = 7.349$, $p < .01$). This significant interaction remained after adding age, gender, ethnicity, maternal education level, past diagnoses of any internalizing or externalizing disorder, medication status and parent-reported CDI score as covariates ($F_{1,32} = 7.58$, $p = .01$). Post hoc analyses performed to identify the source of this interaction revealed decreased positive connectivity within the C-MDD ($p = .014$) group compared to controls, as well as a trend for decreased positive connectivity within the maternal MDD group compared with controls ($p = .058$) . However, there were no significant ($p = .538$) differences in positive connectivity between the control and CM-MDD groups (Figure 2b).

Current Depression Severity

One important question is whether the relationship between either diagnosis of MDD in early childhood or risk for MDD was mediated

FIGURE 2 Mean connectivity between the amygdala and negative and positive networks for all four groups. Note: Figure depicts mean functional connectivity between the amygdala and the (A) negative network and (B) positive network in each of the four groups. Control = children with neither maternal (M) nor childhood-onset (C) major depressive disorder (MDD) history; CM-MDD = children with both maternal and childhood-onset MDD history. *$p < .05$; †$p < .10$.
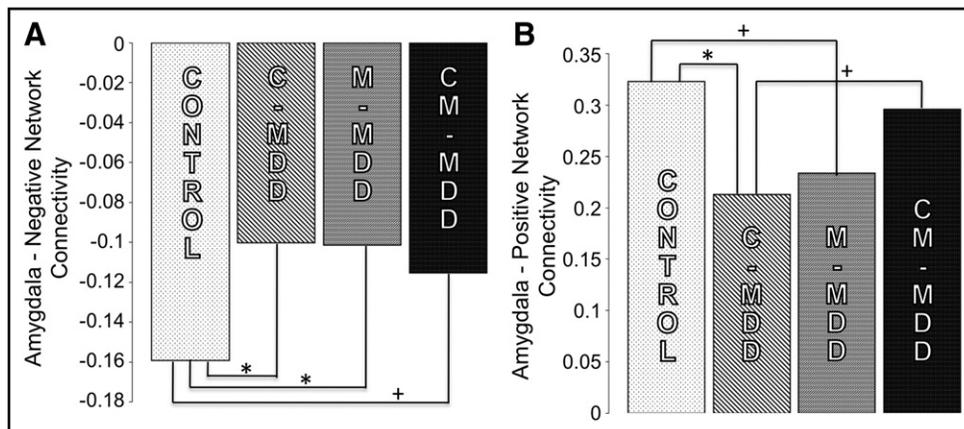
TABLE 2 Correlations Between Depression Severity and Positive (POS) and Negative (NEG) Amygdala Connectivity

Sum Score	All Participants		Maternal History		No Maternal History	
	POS	NEG	POS	NEG	POS	NEG
T1 depression sum score	−0.22	0.22	0.10	−0.07	−0.52**	0.47*
Mean depression sum score	−0.13	0.20	0.20	−0.05	−0.39*	0.32†

Note: $*p < .05$, $**p < .01$, $†p < .10$.

through depressive symptomatology and impairment at the time of scan. To investigate this question, we examined the correlations between both parent and child CDI scores and the mean connectivity for each network (positive or negative). None of these correlations were significant (all $p > .09$).

Relationship to MDD Severity
To determine whether differences in positive and negative amygdala connectivity were associated with individual differences in MDD severity, we computed correlations of the mean positive and negative networks connectivity with MDD severity at the first assessment and mean MDD severity across all assessments (Figures S1 and S2, available online; Table 2). None of these correlations were significant in the sample as a whole, or when the sample was split by the presence or absence of child MDD. However, when the group was split based on the presence or absence of maternal affective disorder history, significant correlations were found within the group with no maternal MDD history. As shown in Figure S2, for children without maternal MDD history, the greater the MDD severity at both baseline and across diagnostic assessments, the lower the connectivity between bilateral amygdala and the regions with which the amygdala is normally positively correlated. A similar finding emerged from correlations with mean negative connectivity, where significant correlations were found only in the group with no maternal history. Mean negative connectivity (Table 2) was significantly positively correlated with MDD sum score at the first interview, and also showed a trend level correlation with mean MDD sum score over all diagnostic time points. Because the expected correlation for the negative network is negative in value, a positive correlation between MDD severity and negative connectivity means that greater MDD severity is associated with reduced negative connectivity be-

tween the amygdala and regions in the negative network.

Relationship to Emotion Regulation
To evaluate the relationship between individual differences in connectivity and emotion regulation we correlated mean negative network connectivity and CEMS sadness dysregulation scores as well as mean positive network connectivity and CEMS sadness coping scores. There were no significant relationships between either network (positive or negative) and sadness inhibition scores. The mean connectivity of the negative network with the amygdala was significantly positively correlated with sadness dysregulation ($r = 0.391$, $p = .009$) indicating that as a child's ability to regulate his/her sadness effectively decreases, so does negative network connectivity. The mean connectivity of the positive network with the amygdala was significantly positively correlated with sadness coping ($r = 0.33$, $p = .031$), indicating that as a child's ability to cope with his/her sadness effectively increases, so does positive network connectivity (Figure 3).

DISCUSSION

Given the deficits in emotion regulation and identification displayed by children with and at increased risk for MDD and the role of the amygdala and associated regions in emotion regulation, the goal of this study was to investigate whether changes in resting state functional connectivity of regions associated with the amygdala are present in children with early-childhood–onset MDD (C-MDD, CM-MDD) and children at increased risk for developing the disease (M-MDD).[61,83] As hypothesized, when compared with controls, we found evidence of reduced connectivity between the bilateral amygdala and the associated negative network

FIGURE 3 Individual differences in childhood emotion management scale scores and negative/positive network connectivity. Note: Scatterplot illustrates the relationship between individual differences in (A) the mean negative network connectivity and Childhood Emotional Management Scale (CEMS) sadness dysregulation scores and (B) the mean positive network connectivity and CEMS sadness coping scores.
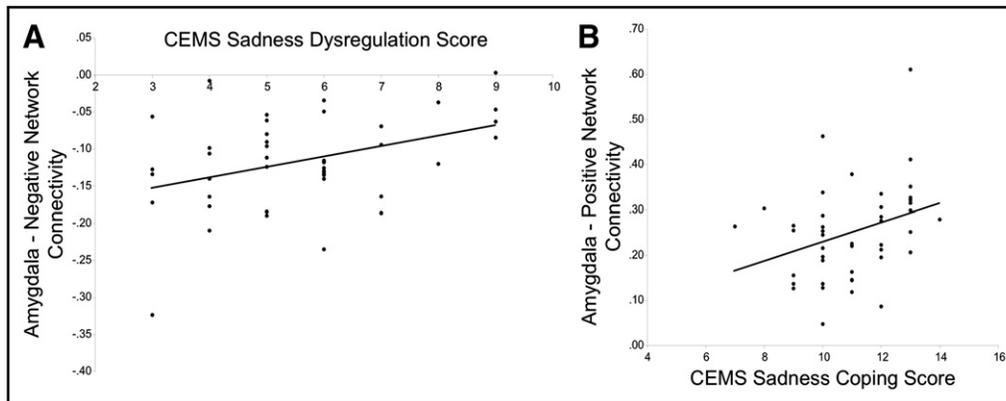
in children with C-MDD, children with a maternal history of MDD or bipolar disorder (M-MDD), and children with both histories (CM-MDD). Children with either early-onset MDD or maternal MDD history displayed reduced connectivity between the amygdala and the positive network compared with controls, whereas children with both histories did not significantly differ from controls. Reduced connectivity for both networks was associated with increased MDD symptom severity only for children without a maternal history of MDD or bipolar disorder. In addition, increased connectivity between the amygdala and the negative and positive networks was associated with reports of increased ability to regulate and cope with sadness, respectively, across all groups.

Connectivity Between Amygdala and Cognitive/Emotion Regulation Regions

As hypothesized, children with C-MDD, a maternal history of MDD/bipolar disorder, or both histories displayed reduced connectivity between the amygdala and negative network. As reviewed above, the negative network consists of regions frequently associated with various aspects of cognitive control and emotion regulation.[84] In addition, stronger connectivity between dorsal frontal regions and the amygdala during emotion regulation is associated with more successful regulation of emotions.[43] Previous work in adults with MDD has found reduced resting state functional connectivity between the amygdala and regions of the prefrontal and

anterior cingulate corticies, interpreted as reflecting alterations in the neural systems normally supporting emotion regulation.[51,53] Also supporting this model, the reduced connectivity among adults with MDD was ameliorated after treatment with antidepressant medication.[51,53] Furthermore, the current findings are consistent with other studies investigating resting state functional connectivity in children with MDD. Gaffrey et al. found reduced connectivity between the subgenual cingulate and prefrontal control regions in children with preschool-onset MDD in a different subset of the same study sample.[59]

Importantly, in our sample, the reduction in connectivity between the amygdala and cognitive control regions was found not only in children who experienced an episode of MDD during the early childhood period (before 8 years of age), but also in those who had never been depressed but were at high risk based on a maternal history of depression. Furthermore, reduced connectivity was associated with reports of a decreased ability to regulate feelings of sadness on the CEMS across all groups. The relationship between negative network connectivity and emotion regulation is consistent with the interpretation that reduced connectivity between amygdala and control regions is associated with poor emotion regulation. Interestingly, for children without a maternal history of MDD or bipolar disorder, reduced connectivity was also associated with more severe depression. However, the finding that the relationship be-

tween MDD severity and negative network connectivity differed as a function of familial risk was unexpected. It is possible that maternal MDD affects how the child's symptom severity is reported or expressed, and thus its relationship to functional connectivity. Further research is needed to confirm and understand this finding.

Taken together, these results suggest that reduction in resting state functional connectivity between the amygdala and the negative network may be a marker of increased risk for MDD and may also be associated with functional indicators of poor emotion regulation. In one sense, our results are somewhat surprising, as one might have expected to see main effects of either personal history of MDD or maternal history, rather than solely an interaction between personal and maternal history. However, the form of the observed interaction was that of an "odd man out," such that children with maternal risk (whether or not they had a personal history) *and* children with a personal history (whether or not they had maternal risk) all showed similarly altered patterns of connectivity. These results suggest that altered patterns of connectivity in the networks that we examined were associated more with risk for depression and did not discriminate between those children who actually developed depression and those who did not. If reduced connectivity of the negative network proves to be a risk factor for MDD, longitudinal studies investigating functional outcome both for children with MDD and those who have not developed the disorder, are needed to determine whether there is a direct relationship between resting state functional connectivity of these networks and future depressive episodes. Moreover, it would be beneficial to determine whether interventions specifically designed to improve emotion regulation skills have any effect on the resting state functional connectivity of these networks, and whether they have any impact on preventing or ameliorating depressive symptoms, particularly in children with reduced connectivity.

Connectivity Between Amygdala and Other Limbic Regions

Children with C-MDD and with maternal history of either MDD or bipolar disorder, but not those with both histories, displayed reduced connectivity between the amygdala and the positive net-

work. As compared with the research on connectivity between the amygdala and cognitive control regions, fewer studies have focused on the functional implications of the strength of connectivity between the amygdala and other limbic regions to which it is positively connected. Thus, there are fewer data upon which to develop hypotheses about alterations in connectivity between the amygdala and limbic system in C-MDD. Increased connectivity between the amygdala and hippocampus has been implicated in increased recall of emotional stimuli over time.[85] As such, the evidence for depressed negative memory bias in depressed individuals suggests that children with MDD could show increased connectivity between the amygdala and positive network.[86] However, Chen et al., in 2008, found reduced positive connectivity between the amygdala and regions such as the hippocampus and parahippocampal gyrus in patients with MDD compared with controls.[51] Our results were more in line with the findings of Chen et al., in that we found reduced connectivity between the amygdala and limbic system in children with either a personal or maternal history of MDD. One possible hypothesis is that this reduction in connectivity could reflect either a failure to integrate emotional experience and memory or a possible inefficiency in using recall of emotional memories as a tool to regulate or cope with emotion. This interpretation would be consistent with our finding that increased connectivity between the amygdala and positive network was associated with reports of better coping with sadness. Unexpectedly, we did not find evidence for such reduced connectivity in the children with both a personal and family history of MDD. This intriguing finding requires further work to clarify the interaction between C-MDD, risk for MDD, and connectivity between the amygdala and other limbic regions.

As noted above, we did not find any connectivity changes selectively associated with personal history of MDD. There are several potential explanations for this. First, it may be that connectivity changes are associated more with risk for depression, and that factors other than connectivity are associated with manifest illness (e.g., amplitude differences in functional responses to emotion processing or emotion regulation, other types of pathophysiological changes). Alternatively, it could be that con-

nectivity changes in different networks that we did not examine (e.g., default mode network) do discriminate between children who develop depression and children who are at risk. Further work in larger samples examining a broader range of brain networks and both connectivity and functional brain reponses will be necessary to distinguish between these possibilities.

The current study suggests that alterations in connectivity, evident in both high-risk groups and those with a very early episode of MDD during the early childhood period of development, may represent an early biological risk marker for MDD. This finding, combined with other alterations in brain functional connectivity in children with MDD onset during the early childhood period, suggests that a neurobiological endophenotype may be evident before the onset of depressive symptoms in childhood- or adolescent-onset MDD.[59,87] Future studies that investigate the longitudinal developmental course of these changes and their plasticity in response to early interventions may prove very fruitful for studies of the prevention and early intervention in MDD.

Interpretation of these results should be examined within the context of several limitations. First, although our study can address important questions regarding functional relationships between key regions involved in emotion processing and regulation in children with or at risk for MDD, our sample size was limited. Larger sample sizes will be needed to confirm and extend our results. Second, our groups were heterogeneous in terms of medication status and ethnicity. A number of the children included in the analyses were medicated at the diagnostic time point before the time of the scan (Table S3, avaliable online). However, we found reductions in connectivity between the amygdala and cognitive control regions both in children with C-MDD and those with only a maternal history of MDD, despite the fact that relatively few children in the maternal history only group were taking any medication. Furthermore, all of the interactions between C-MDD and maternal history of MDD remained significant when controlling for medication status before the scan, as well as when only examining children who were not medicated at that diagnostic time point. Thus, we think that it is unlikely that our results were substantially influenced by medication effects. Also, although ethnicity was included as a cova-

riate in our analyses, our groups did differ in ethnic composition, such that the C-MDD group had fewer children of white ethnicity than the other groups. However, this group did not display a different pattern of connectivity from either the M-MDD and CM-MDD groups, neither of which differs from the other or the control group in terms of ethnicity.

Third, our diagnostic information and group categorization was based on prior assessments and reports of maternal affective disorder history, and did not take into account paternal affective disorder history or current diagnostic status at the time of the scan. Although research suggests that maternal MDD history conveys stronger risk than paternal MDD history, future work investigating the influence of familial MDD history on resting state functional connectivity of the amygdala would benefit from a more complete assessment affective disorder history in both parents.[88] In addition, our group of children with a personal history of MDD were heterogenous in terms of the number of prior MDD episodes and the types of comorbid disorders. Although the group differences in functional connectivity remained even when controlling for these individual differences, it will be important to examine their influence on functional connectivity in future work with larger samples. Finally, future work is needed to characaterize the developmental course of functional connectivity of the amygdala in healthy, typically developing children. Our amygdala networks were defined using an independent dataset from adults rather than in the heatlhy control child sample, so as not to bias us toward finding difference between the MDD and control children. However, it is possible that somewhat different networks of regions showing either positive or negative connectivity with the amygdala would be identified in a child sample, which should be explored in future studies.

In our sample of children 7 through 11 years of age, reduced connectivity within networks both positively and negatively correlated with the amygdala was associated with both C-MDD and a maternal history of the disease. Moreover, reduced connectivity of the negative and positive networks was associated with reports of decreased capacity to regulate and cope with feelings of sadness, respectively. Future studies investigating the relationship

between the resting state functional connectivity of these networks and clinical outcome are needed to determine whether connectivity of these networks has any predictive or diagnostic power, potentially indicating treatment responsivity in children with MDD and the development of future MDD in children at risk for this disorder. &

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TABLE S1 Regions With Positive Connectivity With the Amygdala

Region	Abbreviation	x	y	z
L superior temporal gyrus	LGTs	−27	6	−32
R putamen	Rpu	25	2	−7
R parahippocampal gyrus	RGH	18	−23	−14
R superior temporal gyrus	RGTs	33	10	−28
R hippocampus	Rhi	34	−26	−14
L inferior temporal gyrus	LGTi	−41	−9	−29
L hippocampus	LHi	−27	−26	−12

Note: L = left; R = right.

TABLE S2 Regions Showing Negative Connectivity With the Amygdala

Region	Abbreviation	x	y	z
L middle frontal gyrus (ant. BA 9)	LGFMa	−37	28	32
L supramarginal gyrus	Lgsm	−53	−50	37
R postcentral gyrus (BA 2)	RGPoC	53	−27	42
R precentral Gyrus (BA 6)	RGPrC	51	−6	32
R inferior parietal lobule (BA 40)	RLPi	44	−42	53
R precuneus (BA 7)	RPCu	−11	−73	42
R middle frontal gyrus (BA 9)	RGFm	32	31	30
L superior frontal gyrus (ant. BA 10)	LGFsa	−30	49	14
L superior frontal gyrus (post. BA 8)	LGFsp	−4	15	49
L inferior parietal lobule (BA 39)	LLPi	−36	−61	40
L precentral gyrus (BA 6)	LGPr	−43	−2	27
L middle frontal gyrus (post. BA 6)	LGFmp	−26	9	51

Note: ant. = anterior; BA = Brodmann area; L = left; post. = posterior; R = right.

TABLE S3 Medication Status of Participant Groups at Diagnostic Time Point Before Scan

	Child Only MDD (C-MDD) n = 13	Maternal Only MDD (M-MDD) n = 11	Child and Maternal MDD (CM-MDD) n = 13	Neither Child nor Maternal MDD (CON) n = 14
Number of children on psychiatric medication at diagnostic time point before scan	4 (30.8%)	2 (18.2%)	3 (23.1%)	2 (14.3%)
Central nervous system stimulants (methylphenidate, lisdexamphetamine, amphetamine)	4	1	2	2
Antipsychotic (risperidone)	0	0	0	1
Selective serotonin reuptake inhibitors (sertraline, escitalopram)	0	2	0	0
Selective norepinephrine reuptake inhibitors (atomoxetine)	0	0	0	1
Anticonvulsant (topiramate)	1	0	0	0
Blood pressure medication (clonidine)	1	0	0	0

Note: MDD = major depressive disorder.

FIGURE S1 Mean connectivity of the amygdala with the negative network and major depressive disorder severity scores. Note: Figure depicts a scatter plot of individual differences in the mean negative network connectivity and depression severity scores (sum of symptoms indorsed on the Preschool-Age Psychiatric Assessment) for A) the first in-person diagnostic time point and B) mean of all three in-person diagnostic time points for no maternal and maternal history groups.
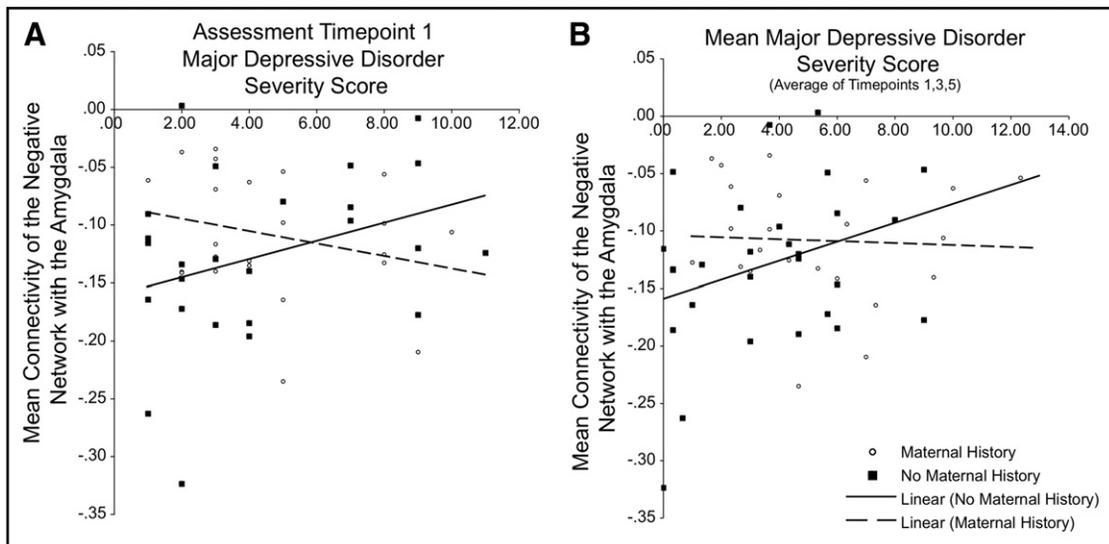
FIGURE S2 Mean connectivity of the amygdala with the positive network and major depressive disorder severity scores. Note: Figure depicts a scatter plot of individual differences in the mean positive network connectivity and depression severity scores (sum of symptoms indorsed on the Preschool-Age Psychiatric Assessment) for A) the first in-person diagnostic time point and B) mean of all three in-person diagnostic time points for no maternal and maternal history groups.