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Amygdala Functional Connectivity Is Associated With Emotion Regulation and Amygdala Reactivity in 4- to 6-Year-Olds

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Objective: Emotion dysregulation has been suggested to be a potent risk factor for multiple psychiatric conditions. Altered amygdala–prefrontal cortex (PFC) connectivity has been consistently linked to emotion dysregulation. Recent data indicate that amygdala–PFC functional connectivity undergoes a prolonged period of development, with amygdala reactivity during early childhood potentially shaping this unfolding process. Little is known about the relationships between amygdala–PFC functional connectivity, amygdala reactivity, and emotion regulation during early childhood. This information is likely critical for understanding early emotion dysregulation as a transdiagnostic risk factor for psychopathology. The current study examined the relationships between amygdala functional connectivity, amygdala reactivity, and emotion regulation in preschoolers.

Method: A total of 66 medication-naive 4- to 6-year-olds participated in a study where resting-state functional magnetic resonance imaging (rs-fMRI) and parent-reported child emotion regulation ability data were collected. fMRI data collected during a face viewing task was also available for 24 children.

Results: Right amygdala–medial PFC (mPFC) functional connectivity was positively associated with child emotion regulation ability and negatively associated with child negative affect and right amygdala reactivity to facial expressions of emotion. Right amygdala–mPFC functional connectivity also statistically mediated the relationship between heightened right amygdala reactivity and elevated child negative affect.

Conclusion: Study findings suggest that amygdala–mPFC functional connectivity during early childhood, and its relationships with amygdala reactivity and emotion regulation during this highly sensitive developmental period, may play an important role in early emotional development. These results inform the neurodevelopmental biology of emotion regulation and its potential relationship with risk for psychopathology.

Key words: irritability, pediatric, emotion regulation, functional connectivity, amygdala

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The ability to effectively regulate negative affect plays a key role in healthy socioemotional development. Failure to do so has been suggested to increase risk for later internalizing psychopathology, including mood and anxiety disorders.[1] Although the temporal ordering of negative affect and emotion regulation remains an area of active research,[2] it has been suggested that elevated negative affect during early childhood may influence the relationship between early disruptions in emotion regulation (ie, difficulty with regulating negative affect) and later internalizing disorders.[3] A large body of research indicates that the amygdala plays an important role in the processing of emotionally salient information,[4] and associations between heightened amygdala reactivity and elevated negative affect during childhood have been established in multiple study samples.[5-7] Recent data have suggested that amygdala connectivity with regions of the prefrontal cortex (PFC) thought to support developing emotion regulation undergo a prolonged period of development. Furthermore, this work has also suggested that early amygdala reactivity may play a key role in shaping the development of the later maturing amygdala–PFC regulatory relationship.[8] As a result, understanding the relationships between amygdala reactivity, amygdala–PFC connectivity, and emotion regulation in early childhood is likely critical for understanding early emotion dysregulation as a transdiagnostic risk factor for psychopathology.[9]

Neurobiological models of mood and anxiety disorders have frequently suggested an altered relationship between putative "top-down" control regions and those considered important for the "bottom-up" generation of emotion. More specifically, these models generally propose altered cortico-limbic connectivity where prefrontal cortical

regions, including the medial PFC (mPFC), fail to regulate and/or respond to evocative signals emanating from limbic structures (eg, amygdala[10]), resulting in high levels of experienced and expressed negative affect. A large body of research in both healthy adults and those with depression and/or anxiety disorders has provided support for the importance of this circuit in emotion regulation.[11-13] In addition, this research has also suggested that altered connectivity within this circuit may play a critical role in the etiology, course, and treatment of mood and anxiety disorders in adolescents and adults.[14,15]

A limited number of studies have investigated amygdala−PFC connectivity and its relationship with emotion regulation and amygdala reactivity during very early development. Nevertheless, age-varying patterns of amygdala−PFC connectivity have been consistently reported. Specifically, Perlman and Pelphrey[16] have reported a positive association between amygdala−ventral mPFC connectivity and child age during an active emotion regulation task. Interestingly, Gee *et al.*[17] have noted that amygdala−mPFC connectivity measured in the presence of negative affect (ie, fear faces) may transition from initially positive during early childhood to increasingly negative as one ages into adulthood. Further, Gabard-Durham *et al.*[18,19] have recently reported that amygdala−mPFC functional connectivity measured at rest becomes increasingly positive as one transitions from childhood into adolescence, and that the strength of this relationship may be shaped by earlier amygdala−mPFC task-based patterns of co-activation. Collectively, these findings suggest that the development of amygdala−mPFC connectivity is already well underway very early in life, and that early amygdala reactivity may play a key role in this process, potentially shaping early emotion regulation development and risk for psychopathology as a result. However, the relationships between amygdala reactivity, amygdala−mPFC functional connectivity, and emotion regulation during very early childhood remain largely unexplored.

To begin filling in this knowledge gap, the current study used resting state functional magnetic resonance imaging (rs-fMRI) and task-based fMRI to investigate the relationships between rs-fMRI amygdala functional connectivity, task-based amygdala reactivity, and emotion regulation in preschool age children. Functional connectivity measured using rs-fMRI has been suggested to reflect the stability and integrity of connections between brain regions, including changes in these features across development.[20] Because a large body of research has consistently supported the importance of the amygdala in cortico-limbic models of emotion regulation and the development of psychopathology,[21] we chose to focus on this structure as an *a priori* region of interest (ROI). Also, in line with previous research suggesting that amygdala−mPFC functional connectivity becomes increasingly positive over development,[22,23] we hypothesized that rs-fMRI functional connectivity between the amygdala and mPFC would be positively related to child emotion regulation ability and negatively related to amygdala reactivity to facial expressions of emotion and child negative affect. Following recent data suggesting that amygdala function might shape amygdala connectivity with mPFC,[8] we also hypothesized that amygdala−mPFC functional connectivity would statistically mediate the relationship between heightened amygdala reactivity and elevated child negative affect.

METHOD

Participants

Children 4 to 6 years old were recruited from pediatrician's offices, daycare centers, and other community resources throughout the greater St. Louis area. To increase sample variance in depressive symptoms, caregivers endorsing items suggestive of "low" ($\leq$1 items) or "high" ($\geq$3 items) risk for mood-related difficulties on the Preschool Feelings Checklist[24] (PFChecklist) were contacted and invited to complete additional phone screening steps regarding their preschoolers. The presence of neurological disorders (eg, seizure disorder, etc), autism spectrum disorders or developmental delays, premature birth (<36 weeks' gestation), and psychotropic medication use were assessed and acted as exclusionary for all children. Children passing the exclusion criteria were invited to enroll in the full study. A total of 132 children were enrolled. Following study enrollment, children and caregivers participated in an initial in-laboratory assessment and a subsequent MRI scan 7 to 10 days later. Of the 132 children, 66 children provided resting state fMRI data passing stringent quality control (QC) measures (50%; see Amygdala Functional Connectivity and Individual Differences in Child Emotion Regulation section below) and are the main focus of this report. Parental written consent and child verbal assent were obtained for all subjects. The Institutional Review Board at Washington University in St. Louis approved all experimental procedures.

Diagnostic Assessment

Diagnostic assessments were conducted using developmentally appropriate, interviewer-based psychiatric interviews designed for use with the primary caregivers of children between 3 and 7 years of age (Supplement 1, available online).

Parent Report Measure of Child Emotion Regulation and Negative Affect

Parents completed the Emotion Regulation Checklist (ERC).[25] The ERC is a parent-report measure of children's dysregulated negative affect (Negativity) and successful emotion regulation (Emotion Regulation), and includes both positively and negatively weighted items to be rated on a four-point Likert scale.

To assess maternal depression, parents filled out the Beck Depression Inventory–II (BDI–II),[26] a validated 21-item measure of depression symptom presence and severity in adults.

Functional Imaging Data Acquisition and Preprocessing

To acclimate children to the scanning environment and to increase the likelihood of acquiring high-quality data, children were shown a video introducing the fMRI experience, exposed to a simulated scanning environment using a mock scanner, and watched a movie of their choice during structural scans.

Imaging data were collected using a Siemens MAGNETOM 3T TIM TRIO (Erlangen, Germany) whole-body system. Image acquisition included an initial low-resolution three-dimensional sagittal T1-weighted MP-RAGE rapidly warped to Talairach space[27] and used to provide on-line slice localization for the functional images, placing them as close as possible to the target template. T1 images (TR = 2,400 milliseconds, TE = 3.16 milliseconds, field of view [FOV] = 256 mm, flip angle = 8°, 176 slices with 1-mm^3 voxels) were acquired as part of a structural imaging protocol and used in the transformation of images to a common template space optimized for preschool children.[27] The accuracy and validity of this transformation for preschool-age children has been demonstrated in previous research[28] and was confirmed through visual inspection for distortions and the accuracy of alignment for key cortical and subcortical landmarks. The functional images were collected with a 12-channel head coil using an asymmetric spin-echo echo-planar sequence sensitive to blood-oxygen level dependent (BOLD) contrast (T2*). Half of the sample included in the current study (n = 33) had data collected using a TR of 2,500 milliseconds (TE = 27 milliseconds, FOV = 256 mm, flip = 90°), whereas the other half had a TR of 2,000 milliseconds (TE = 27 milliseconds, FOV = 256 mm, flip = 90°), resulting in sets of 36 and 34 contiguous axial images with isotropic voxels (4 mm^3) acquired parallel to the anterior−posterior commissure plane collected during each functional run, respectively. Children were instructed to rest quietly and to look at a fixation cross as functional images were acquired. Three resting state functional MRI runs of 76 or 95 TRs (3 minutes 10 seconds per run) were collected for each child.

Amygdala Reactivity to Facial Expressions of Emotion

In addition to completing three resting fMRI runs, a subset of 24 children also completed an fMRI task investigating brain activity while viewing facial expressions of emotion. In brief, children were presented with a series of faces varying in affective content and asked to complete a simple button press each time a face appeared. Study analyses found a cluster of right amygdala reactivity to facial expressions of emotion (including sad, happy, fear, and neutral expressions) that was greater in preschoolers with depression relative to those without, and that was also positively correlated with child negative affect. Given the current study's interest in the relationships between amygdala functional connectivity, amygdala reactivity, and emotion regulation, individual magnitude estimates of right amygdala reactivity to facial expressions of emotion versus baseline fixation were calculated using the right amygdala cluster identified in this previous study (see Supplement 1, available online, and Gaffrey *et al.*[5] for additional task and task-related amygdala ROI details). All subsequent analyses including right amygdala reactivity make use of these data.

Amygdala Functional Connectivity and Individual Differences in Child Emotion Regulation

Before preprocessing, the first 10 seconds of data acquisition (2.5-second TR group, 4 frames; 2-second TR group, 5 frames) of each run were discarded to allow for signal stabilization. The fMRI data were preprocessed and analyzed using in-house Washington University software and included the following steps: (1) correction for potential slice intensity differences due to interleaved acquisition; (2) normalization across runs by scaling whole-brain signal intensity to a fixed value of 1,000; (3) correction for head movement in and across runs; and (4) co-registration to Talairach space using a 12-parameter linear (affine) transformation. Voxels were resampled to 3 mm^3 during a single cubic spline interpolation that combined steps 3 and 4 above.

To protect from spurious correlations unlikely to reflect neural activity, additional rs-fMRI specific preprocessing steps were carried out following the "scrubbing" procedure previously described by Power *et al.*[29] Briefly, a temporal mask that identified motion-contaminated frames was created for each subject and subsequently applied during rs-fMRI preprocessing. Motion-contaminated frames were identified using frame-to-frame displacement (relative FD, calculated as the sum of absolute values for the three translational and three rotational motion parameters

identified during initial fMRI preprocessing). Frames with relative FD >0.25 mm were identified and ignored during rs-fMRI preprocessing and all correlation calculations. In addition, frames with relative FD ≤0.25 mm but not included in contiguous chunks of 5 or more frames passing the relative FD ≤0.25-mm threshold were similarly ignored. rs-fMRI preprocessing included the following: (1) multiple regression of nuisance signals including the 6 rigid body motion parameters for each frame and the frame before it as well as their squares (24 total motion parameters), ventricle signal, deep white matter signal, and whole-brain signal and the first-order temporal derivatives of these signals; (2) temporally filtering BOLD images (0.009−0.08 Hz); and (3) spatial smoothing with a 6-mm full-width at half maximum (FWHM) Gaussian filter. Ventricles, deep white matter, and whole-brain signal were extracted using child-specific ROI masks created using FreeSurfer 5. After postprocessing, children were required to have 4 minutes or more of rs-fMRI data to be included in study analyses. Average relative FD (measured as root mean squared [RMS]) after postprocessing was 0.13 mm (0.02 mm) and number of frames remaining after scrubbing was 157 (±31; average, 6.5 minutes) for children with 2.5-second TR and 189 (±45; average, 6.3 minutes) for children with 2-second TR.

After preprocessing of rs-fMRI data, the BOLD time courses for the right and left amygdala were extracted for each child (N = 66) using *a priori*−defined ROI adapted from Roy *et al.*[30] The correlation coefficients between each amygdala ROI and all other voxels in the brain were then calculated. The Fisher *r* to *z* transform was applied to each map, and subsequent analyses used these transformed data. Relationships between amygdala connectivity and child emotion regulation ability were identified using custom software (http://www.nil.wustl.edu/~fidl/). Specifically, the connectivity between each amygdala ROI and the rest of the brain as a function of child emotion regulation ability was identified using a standard whole-brain correlation approach. The necessary voxel *z*-value/cluster size combination for a corrected false positive rate of .05 ($z \geq 2.25$ + cluster size thresholding [63 voxels]) was determined using the Analysis of Functional Neuro-Images (AFNI) 3dFWHMx and 3dClustSim commands including the acf option.[31]

Amygdala Reactivity, Amygdala−mPFC Connectivity, and Negative Affect

Individual relationships between amygdala reactivity, amygdala−mPFC connectivity, and child negative affect were examined using separate correlational analyses with a two-tailed approach to significance (IBM SPSS Statistics version 21; SPSS Inc., Chicago, I). To test our *a priori* hypothesis that amygdala−mPFC connectivity statistically mediates the relationship between heightened amygdala reactivity and child negative affect in preschoolers, we used the PROCESS macro procedure for SPSS. The current statistical mediation analyses focused on the 24 preschoolers who had amygdala functional connectivity, amygdala reactivity, and negative affect data available. Following Hayes,[32] a significant effect of statistical mediation would indicate that the association between amygdala reactivity and negative affect is statistically mediated by amygdala−mPFC connectivity. A multivariate approach to identifying potential outliers using Mahalanobis D^2 was conducted prior to carrying out our *a priori* correlational and mediation analyses. No outliers were identified.

RESULTS

Demographic and Child Characteristics

Child demographic, emotion regulation, and negative affect characteristics and maternal depression scores are summarized in Table 1. When compared based on acquisition group (ie, 2.5-second versus 2-second [s] TR), ERC Negativity (2.5s = 27; 2s = 24.7) and Emotion Regulation (2.5s = 26; 2s = 26.9) did not differ between groups. However, the 2.5s TR group was slightly younger (63.6

TABLE 1 Study Group Characteristics (N = 66)

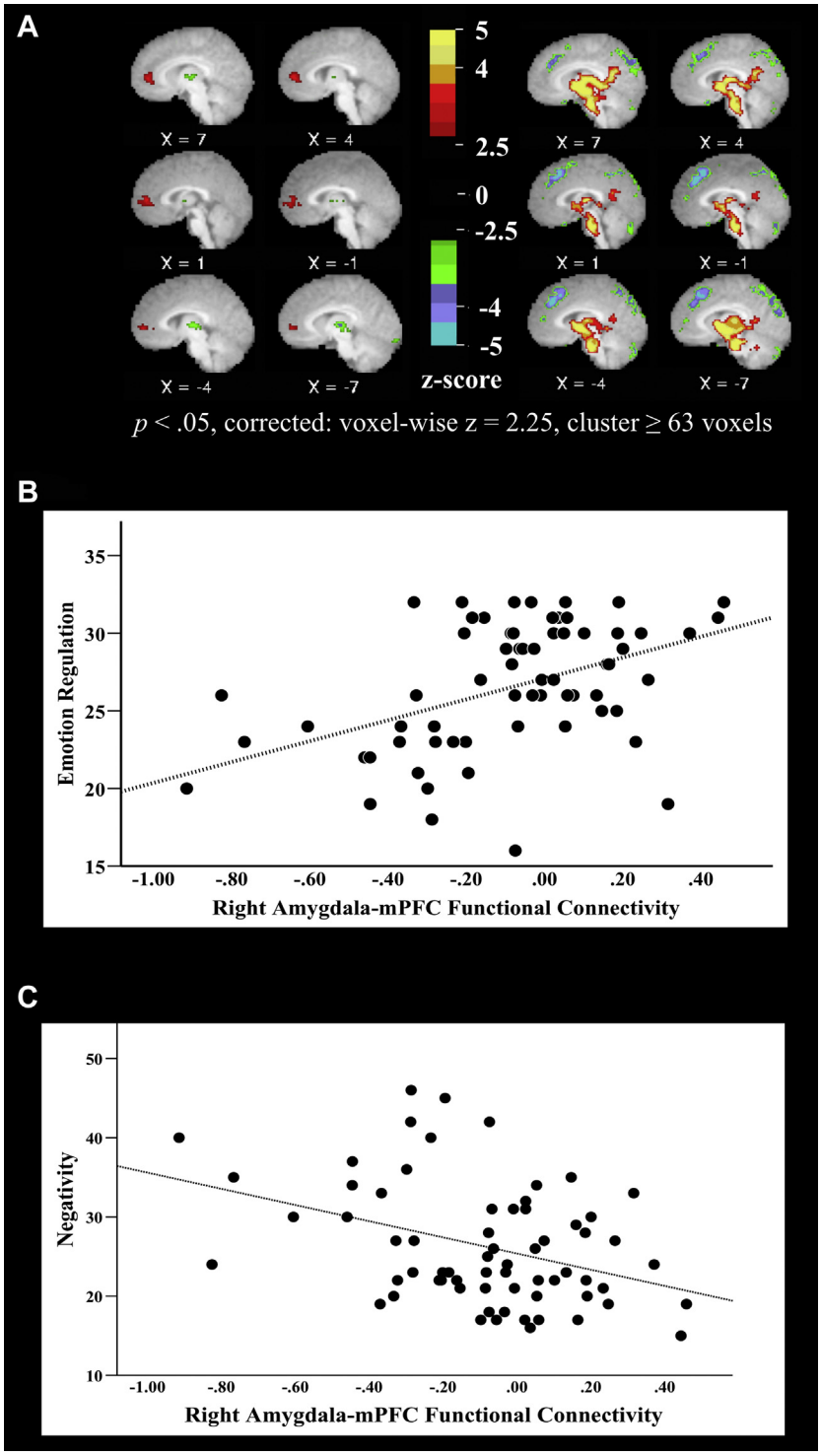
Characteristic	Value
Age, mo	66.1 (±9.7)
Sex, female/male	34/32
Ethnicity, White/AA/other	47/14/5
PFC Screen[a], low/high	41/25
ERC Negativity	26.3 (±7.6)
ERC Emotion Regulation	26.5 (±4.2)
Diagnoses[b]	
None	43
Internalizing	16
Externalizing	3
Internalizing and Externalizing	4

Note: AA = African American; ERC = Emotion Regulation Checklist; PFC = Preschool Feelings Checklist.

[a]*Number of children with caregiver reporting "low" (≤1 PFC items endorsed) or "high" (≥3 PFC items endorsed) levels of depressive symptoms during initial screen.*

[b]*Internalizing: preschool depression (n = 6), preschool depression and anxiety disorder (n = 10); externalizing: oppositional defiant disorder (n = 1), attention-deficit/hyperactivity disorder (n = 2); internalizing and externalizing: preschool depression, anxiety disorder, oppositional defiant disorder, conduct disorder, and attention-deficit/hyperactivity disorder (n = 1), preschool depression and attention-deficit/hyperactivity disorder (n = 1), preschool depression and oppositional defiant disorder (n = 2).*

FIGURE 1 Regions Identified as Functionally Connected With the Right Amygdala



Note: *Right amygdala functional connectivity when using a (A) whole-brain correlational approach including parent-reported child emotion regulation ability (leftmost brain images) or a whole-brain group approach to identify regions (rightmost brain images). Functional connectivity between the amygdala and medial prefrontal cortex (mPFC) was found only when using the whole-brain correlational approach that included child emotion regulation ability. (B) Scatter plot depicting the relationship between child emotion regulation (values from Emotion Regulation Checklist) and right amygdala–mPFC functional connectivity (z-transformed r values). This plot is for illustration purposes only, given that child emotion regulation scores were used to identify the right amygdala's functional connectivity with the mPFC. (C) Scatter plot depicting the relationship between child negative affect (values from Emotion Regulation Checklist) and right amygdala–mPFC functional connectivity (z-transformed r values). Warmer colors in the z-score scale indicate positive scores, and cool colors indicate negative scores. Please note color figures are available online.*

[10.3] months) than the 2s TR group (68.5 [8.6] months; $t = -2.1$, $p = .04$). Average relative FD was not associated with ERC Negativity ($r = 0.03$, $p = .7$), Emotion Regulation ($r = -0.01$, $p = .9$), right amygdala–mPFC functional connectivity ($r = -0.17$, $p = .17$), or right amygdala reactivity to faces ($r = -0.03$, $p = .9$). In line with previous research indicating that maternal mood state likely inflates parent report of child psychopathology, there was a significant positive correlation between Negativity and BDI-II scores ($r = .45$, $p < .001$) in the current sample. Thus, all analyses including Negativity controlled for maternal BDI-II scores.

Neuroimaging Findings

Amygdala Connectivity. Whole-brain right and left amygdala connectivity results are illustrated in Figure S1, Figure S2, and Table S1, available online. In brief, both the left and right amygdala showed widespread patterns of functional connectivity similar to those previously reported for older age groups, including positive functional connectivity with other subcortical regions including the hypothalamus and thalamus and negative functional connectivity with cortical regions including dorsolateral prefrontal cortex, dorsal anterior cingulate, and intraparietal sulcus. However, functional connectivity between the mPFC and either the right or left amygdala was absent (Figure 1A).

Amygdala Connectivity and Child Emotion Regulation. Although absent at the group level, whole-brain correlational analyses (N = 66) revealed a positive relationship between individual difference emotion regulation scores and right amygdala connectivity with the mPFC (Table 2 and Figure 1A and B). In addition, negative correlations between emotion regulation and right amygdala connectivity with left cerebellum, right fusiform gyrus, and right and left thalamus were also identified (Table 2). Given our *a priori* hypotheses focused on amygdala–mPFC functional connectivity, right amygdala–mPFC functional connectivity individual difference scores used in subsequent mediation analyses were calculated for each child using the identified mPFC cluster (Figure 1A). Importantly, right amygdala–mPFC functional connectivity was not correlated with the number of available rs-fMRI frames ($r = 0.16$, $p = .19$).

No significant correlations between left amygdala–whole-brain connectivity and emotion regulation scores were identified.

Amygdala Reactivity, Amygdala–mPFC Connectivity, and Negative Affect. Following our *a priori* hypotheses, in the full sample (N = 66) right amygdala–mPFC connectivity was negatively correlated with Negativity ($r = -0.38$, $p = .002$) (Figure 1C). This relationship remained significant when age (in months), sex, and fMRI acquisition TR group (ie, 2 versus 2.5 seconds) were controlled (amygdala–mPFC connectivity–Negativity: partial $r = -0.37$, $p = .003$) and when maternal depression was added as an additional covariate (partial $r = -0.39$, $p = .002$).

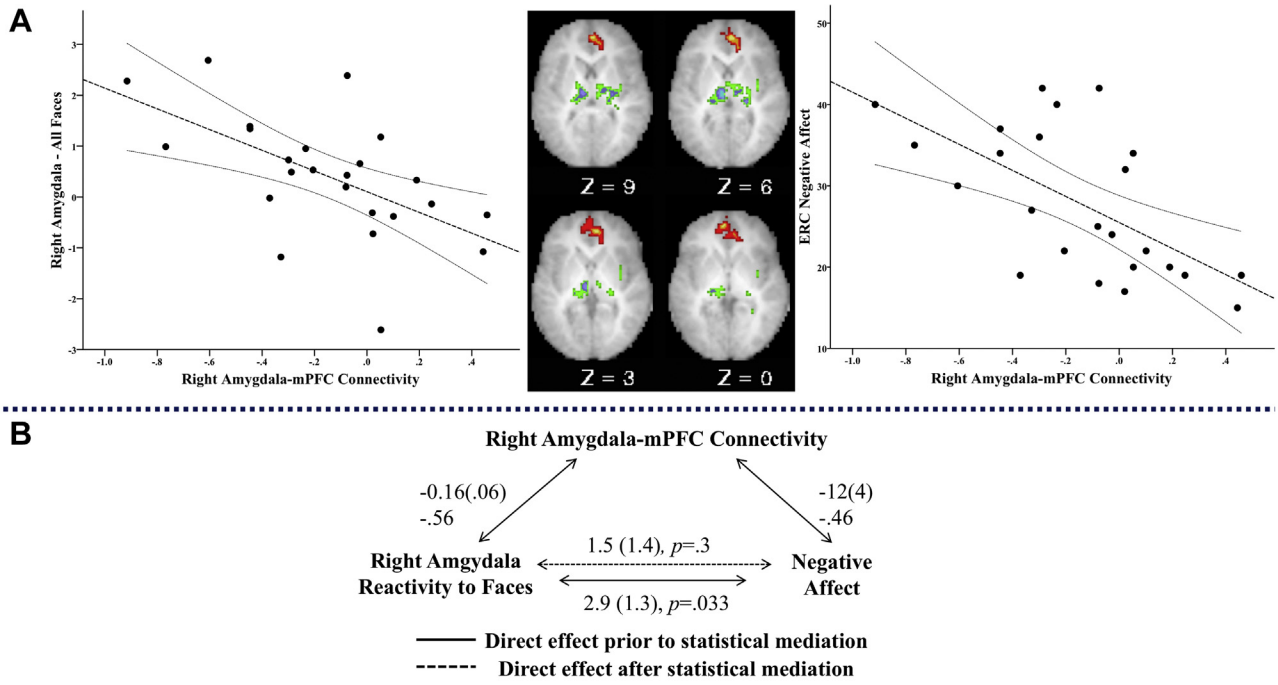
In our subgroup with amygdala functional connectivity and reactivity data (n = 24), right amygdala–mPFC functional connectivity was negatively correlated with child amygdala reactivity ($r = -0.58$, $p = .003$) and Negativity ($r = -0.62$, $p = .001$) (Figure 2A). These relationships remained significant when age (in months) and sex were controlled (amygdala–mPFC connectivity–amygdala reactivity: partial $r = -0.56$, $p = .007$; amygdala–mPFC connectivity–Negativity: partial $r = -0.625$, $p = .005$) and when maternal depression was added as additional covariate (amygdala–mPFC connectivity–amygdala reactivity: partial $r = -0.51$, $p = .02$; amygdala–mPFC

TABLE 2 Regions Identified as Significantly Connected With Amygdala as a Function of Child Emotion Regulation Ability During Whole-Brain Correlational Analyses

			Coordinates				
Region	**Hemisphere**	**BA**	**X**	**Y**	**Z**	**Cluster (voxels)**	**Valence**
Right amygdala ROI	R		26	−7	−15		
				Peak Voxel			
Cerebellum	L		−23	−85	−21	94	−
Fusiform gyrus	R		22	−49	−11	130	−
Medial prefrontal cortex	R	32	3	43	4	164	+
Thalamus	R		22	−19	10	190	−
Thalamus	L		−11	−22	4	116	−
Left amygdala ROI	L		−21	−7	16		
No significant clusters							

Note: BA = Brodmann area; L= left; R = right; ROI = region of interest.

FIGURE 2 Relationships Between Right Amygdala-Medial Prefrontal Cortex (mPFC) Functional Connectivity, Right Amygdala Reactivity, and Negative Affect



Note: (A) Scatter plot illustrating the relationship between right amygdala–mPFC functional connectivity and right amygdala reactivity to facial expressions of emotion and parent-reported negative affect (see Methods for greater detail). (B) Results of statistical mediation model in which amygdala–mPFC functional connectivity was found to mediate the relationship between amygdala reactivity and child negative affect. The association between right amygdala reactivity and right amygdala–mPFC functional connectivity, and between right amygdala–mPFC functional connectivity and Negativity, report unstandardized (top value) and standardized (bottom value) β values after controlling for sex, age, and maternal depression. Brain images in the center of the figure illustrate the mPFC region used in these analyses. Please note color figures are available online.

connectivity–Negativity: partial $r = -0.589$, $p = .005$). In addition, right amygdala reactivity was positively correlated with Negativity ($r = 0.63$, $p = .001$) (Figure 2A) and the relationship remained significant after controlling for age and sex (partial $r = 0.61$, $p = .003$) and when maternal depression was added as an additional covariate (partial $r = 0.47$, $p = .03$). Reduced right amygdala–mPFC functional connectivity scores were found to fully statistically mediate the positive relationship between right amygdala reactivity and Negativity scores when age and sex were controlled (PROCESS Indirect Effect [10,000 bootstrap samples]: 1.6 (1.1), bias-corrected 95% CI: 0.15/4.3) and when maternal depression was included as an additional covariate (PROCESS Indirect Effect [10,000 bootstrap samples]: 1.6 (1.1), bias-corrected 95% CI: 0.1/4.4) (Figure 2B). An alternative model testing right amygdala reactivity as a statistical mediator of the relationship between right amygdala–mPFC functional connectivity and Negativity and including age, sex, and maternal depression as covariates was not significant (PROCESS Indirect Effect [10,000 bootstrap samples]: −3 (3), bias-corrected 95% CI: −11.1/1.9). An additional model testing right amygdala–mPFC task-based connectivity (see Supplement 1, available online, for task-based connectivity methods) as a statistical mediator of the relationship between right amygdala reactivity and Negativity and including age, sex, and maternal depression as covariates was not significant (PROCESS Indirect Effect [10,000 bootstrap samples]: −0.46 (1), bias-corrected 95% CI: −2.7/1.5).

DISCUSSION

The current study used fMRI to investigate the relationships between amygdala connectivity, amygdala reactivity, and emotion regulation in preschoolers. Consistent with predictions, we found that right amygdala–mPFC functional connectivity was positively associated with emotion regulation in preschoolers. We also found that right amygdala–mPFC connectivity was negatively related to right amygdala reactivity and negative affect. Importantly, in our smaller subsample, amygdala–mPFC connectivity was found to statistically mediate the relationship between heightened right amygdala reactivity and elevated child negative affect. The current findings are consistent with

previous research indicating a relationship between amygdala−mPFC functional connectivity and emotion regulation.[18] They also provide novel evidence supporting a potential role for early amygdala reactivity in the developing relationship between amygdala−mPFC functional connectivity and emotion regulation during the preschool period.

Amygdala−mPFC functional connectivity has been suggested to undergo a prolonged period of development, becoming increasingly positive as one transitions from childhood into adolescence and adulthood.[18] Nevertheless, findings from previous studies investigating amygdala−mPFC functional connectivity during early childhood have been mixed. More specifically, amygdala−mPFC functional connectivity in very young children has been reported as positive as well as absent.[18,33-35] Importantly, and potentially helping to explain these mixed results, it has also been reported that functional connectivity between these two regions may be either influenced by or reflective of other child-specific features or experiences.[33,36] In line with these previous findings, the current study did not find statistically significant amygdala functional connectivity with the mPFC when examined at the group level. However, amygdala−mPFC functional connectivity was found to be positively correlated with child emotion regulation ability. Combined, these findings highlight an important distinction between developmental variation across chronological age (eg, child to adult) and within a specific developmental period (ie, the preschool period). More specifically, the current findings suggest that increased individual level variability in a skill related to amygdala−mPFC functional connectivity may result in the absence of statistically identifiable (ie, stable) patterns of group level connectivity. Emotion regulation and associated skills (eg, inhibition) are highly variable in young children and undergo a rapid period of development during the preschool years.[2,37] Longitudinal research suggests that although overall skill level in these areas increase with age, the relative rank of group members may remain consistent, potentially preserving dimensional relationships with related skills.[38] In light of this, the reported absence of group-level right amygdala−mPFC functional connectivity in the current study may be reflective of greater variation in the strength of this connection as it relates to rapid emotion regulation development during early childhood rather than an expected "absence" of connectivity between these two structures at this age. As such, it is possible that age-expected patterns of group level stability in amygdala−mPFC functional connectivity (ie, group-level means) reported in previous research may partly reflect greater variability in emotion regulation skill at one age versus another as well as maturational change when compared across age groups. Future studies investigating the development of amygdala−mPFC functional connectivity should carefully consider whether differences in behavioral and/or environmental variability between age groups may be contributing to identified patterns of age-related change in connectivity. Unfortunately, the current data cannot inform the ongoing maturation of amygdala−mPFC functional connectivity in the current sample (ie, changes in amygdala−mPFC connectivity strength and valence as the children age) and how it may or may not interact with and/or influence developing emotion regulation. However, similar to previous research in older age groups,[39] the current findings do suggest that individual differences in amygdala−mPFC connectivity and emotion regulation are already present during this very early period of development, and that longitudinal studies will be necessary to better understand the changing nature of this relationship as children age.

The current study provides unique insight into how amygdala−mPFC functional connectivity and emotion regulation are related to each other in preschool-age children. More specifically, it suggests that increasingly positive amygdala−mPFC functional connectivity is associated with stronger emotion regulation skill at this age. Previous research has pointed to the preschool period as a rapid period of emotion regulation development.[2] It has also suggested that age-related changes in amygdala−PFC connectivity may be related to developing emotion regulation.[7,40] However, this work has not directly tested the relationship between amygdala functional connectivity and emotion regulation in a large group of preschool-age children. As such, the current study provides novel support for theoretical models suggesting that amygdala−mPFC functional connectivity is critically related to the ongoing development of emotion regulation during early childhood.[8] It also raises the intriguing possibility that amygdala functional connectivity may prove useful for further delineating how specific neural circuits and emotion regulation are related in very young children, and whether well-validated treatments shown to increase emotion regulation skill in clinical populations work by positively altering these circuits.[41] However, the current findings cannot directly inform whether the relationship between amygdala−mPFC functional connectivity and emotion regulation is a function of the amygdala, the mPFC, developing emotion regulation influencing amygdala−mPFC functional connectivity, or some combination thereof. They also cannot establish a causal relationship or identify the potential influence of environmental factors (eg, caregiving) on the relationship between amygdala−mPFC functional connectivity and emotion regulation. Thus, large longitudinal studies spanning multiple developmental periods and measuring cortico-limbic functional connectivity, developing emotion regulation, and important environmental factors will be needed and are a logical and critical next step.

Negative affect is a prominent symptom in multiple psychiatric diagnoses (eg, major depressive disorder, anxiety disorders, etc[42]). In line with this, a growing body of research has suggested that elevations in negative affect during early development may function as an important marker of risk for later psychiatric illness. For example, elevated negative affect during preschool has been found to significantly increase the odds of receiving a diagnosis of depression during later school-age and adolescence.[43,44] Recent data also indicate that elevated negative affect during early childhood is associated with heightened amygdala reactivity[5] and that amygdala reactivity may play a key role in shaping functional connectivity between the amygdala and mPFC.[19] As a result, individual differences in amygdala reactivity during early childhood have been suggested to mechanistically alter maturing patterns of connectivity between the amygdala and other regions important for emotion regulation, potentially placing an individual at greater or lesser risk for psychopathology.[8] In line with this, the current study found that right amygdala−mPFC functional connectivity statistically mediated the relationship between heightened right amygdala reactivity and elevated negative affect in the current sample even after controlling for age, sex, and maternal depression. Laterality of amygdala function remains an area of open investigation. However, previous research has suggested that right amygdala reactivity is preferentially involved in the rapid detection of emotionally relevant stimuli.[45] Thus, the right amygdala may be particularly well suited for directing attention toward salient information that "shapes" the focus of more elaborated processing within the mPFC. Over time, with greater experience and maturation of reciprocal connections between the amygdala and mPFC, goal-related modulation of amygdala reactivity by the mPFC may become the more predominant pattern. However, given that concurrent data were used to examine whether right amygdala−mPFC functional connectivity statistically mediated the relationship between amygdala reactivity and negative affect, significant continued testing of the specificity and maturation of these suggested patterns of relationships is required. Nevertheless, when viewed in light of previous research indicating that early amygdala function shapes later amygdala−mPFC connectivity,[8,19] the current findings support the developing relationship between amygdala function and functional connectivity with the mPFC as a potential intermediate phenotype capable of informing common and/or unique etiological pathways associated with later psychopathology.[46]

Several limitations should be considered. Our sample size of 66 preschoolers, although relatively large given the young age of our participants, may have limited our statistical power to identify other meaningful relationships. Given our interest in individual differences, reducing well-documented effects of subject motion on patterns of functional connectivity was of primary concern.[47] Global signal regression (GSR) in combination with frame censoring is one of the top-performing models for mitigating the distance-dependent profile of motion artifact found in rs-fMRI.[48,49] However, GSR has been reported to shift the distribution of functional connectivity scores (ie, becomes zero centered). The reported findings should be viewed with this limitation in mind. The small sample size included in our statistical mediation analysis requires this finding to be replicated in a larger group. The standardized β values provided in Figure 2 may prove useful for informing the sample size of future studies.[50] In addition, given that all measures were taken concurrently, the current results cannot inform directions of causality. Nevertheless, the current findings inform the neurodevelopmental biology of emotion regulation and further underscore early childhood as an important developmental period for understanding its earliest roots and potential contribution to risk for psychopathology.

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