



Amygdala Resting Connectivity Mediates Association Between Maternal Aggression and Adolescent Major Depression: A 7-Year Longitudinal Study

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Objective: The parent–adolescent relationship is an important predictor of adolescent mental health, especially depressive disorders. This relationship is constructed in the context of maturing emotion neurobiology and could help shape such neurobiology in ways that are important for current and future mental health. Amygdala resting-state functional networks have been linked to depression, but whether such resting connectivity is associated with parent affective behaviors or acts as a salient mediator between parenting and risk for depressive disorder is unknown.

Method: In the present study of 128 individuals, a 7-year longitudinal design was used to examine how observed maternal aggressive behavior during mother–adolescent interactions in early adolescence (12 years) predicted amygdala (whole and subregion)-based resting connectivity in mid adolescence (16 years). In 101 of those participants, whether altered amygdala resting-state connectivity mediated the association between maternal aggressive behavior and the first onset of major depressive disorder (MDD) in late adolescence (19 years) was analyzed.

Results: Maternal aggression was related to resting-state functional connectivity between the amygdala and right superior temporal-posterior insula-Heschl gyri, bilateral visual cortex, and left temporal and insula cortices (the latter being driven by the centromedial amygdala subregion; $p < .001$). Further, amygdala and centromedial amygdala connectivity with the temporal and insula cortices mediated the association between maternal aggression and late adolescent-onset MDD (CI 0.20 to 2.87; CI 0.13 to 2.40, respectively).

Conclusion: These findings are consistent with previous literature documenting the importance of amygdala resting networks for adolescent depression but further suggest the importance of parental affective (particularly aggressive) behavior in the development of such functional connectivity patterns during this period of peak onset for mental health disorders.

Key words: amygdala, resting state functional connectivity, adolescent, maternal aggression, parent–adolescent relationship

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The transition from childhood to adolescence represents a significant shift in the role of parents, whereby adolescent autonomy and peer influences need to be renegotiated, often resulting in parent–adolescent conflict.^{1,2} The affective climate within the parent–adolescent relationship is a strong predictor of adolescent mental health. Specifically, parental expression of emotion and aversive parent–adolescent relationships are risk factors for adolescent depression and other mental illnesses.^{3–5} Such parental emotion expression is a form of emotion socialization, which shapes the emotional climate within the family and influences the emotional development of children through processes such as modeling and social referencing. Parental expression of negative, angry, or aggressive emotions in

particular has been associated with adolescent depression.^{5–7} We previously found that maternal expression of aggressive behavior that is “out of context” (i.e., expressed during a positive interaction task) prospectively predicts the onset of adolescent major depressive disorder (MDD),⁸ suggesting that such behavior can be particularly maladaptive.

Parental socialization of adolescent emotions occurs in the context of significant structural and functional changes taking place in the adolescent’s brain^{9–12} and could help to shape the maturation of developmentally plastic neural networks with consequences for emotion regulation (e.g.,^{13,14}). In particular, the amygdala undergoes significant structural and functional development throughout adolescence, which is related to adolescent emotionality.^{12–15} Importantly, recent evidence suggests that parental expression of negative emotion shapes a child’s amygdala reactivity during affective processing.¹⁶

Amygdala resting-state functional brain networks also undergo significant developmental change¹⁷ and are associated with affective processing^{18,19} and depression in



Supplemental material cited in this article is available online.

adolescents. For instance, amygdala resting-state functional connectivity with the temporal pole²⁰ and medial prefrontal cortex²¹ is increased in adolescents with elevated depression symptoms, whereas resting-state functional connectivity between the amygdala and hippocampus and parahippocampal cortex²² and between the amygdala and posterior insula²³ is lower in adolescents with increased depressive symptoms. Importantly, parenting factors appear to regulate the development of resting-state functional networks in the amygdala in infants,^{24,25} which could be an additional neurobiological pathway linking parenting to mental health. However, whether parenting (and particularly parental expression of negative emotion) in adolescence specifically influences distinct amygdala resting-state networks in ways that relate to risk for adolescent depression is unknown.

During the past decade, appreciation that the amygdala consists of distinct and integrated functional subdivisions has increased. Although classically considered a single structure, functional and histologic evidence points to a broad subdivision within the amygdaloid complex encompassing the centromedial (CM), laterobasal (LB), and superficial (SF) nuclear groups.^{26,27} Broadly, the CM group is involved in the perception of emotions and the generation of behavioral responses, the LB group facilitates associative learning, and the SF group is implicated in olfaction.²⁸ Appreciation for the role of different amygdala subdivisions in adolescent depression and the responsiveness of these different subdivisions to environmental contexts (such as parental aggressive behavior) are not well understood.

The aim of the present study was to longitudinally assess the association between maternal expression of aggression during the early adolescent period and amygdala resting-state connectivity (for the whole amygdala and within the 3 subdivisions) later during mid adolescence and to investigate whether these parenting brain associations predict the subsequent emergence of depressive disorder in later adolescence, a high-risk period for depression onset.²⁹

We hypothesized that increased maternal expression of aggressive behavior toward adolescents would be associated with amygdala-seeded network connectivity in emotion regulation and processing regions (e.g., prefrontal, insula, and temporal cortices), and that connectivity with these regions would mediate the association between aggressive maternal behavior and subsequent first-onset depression in late adolescence.

METHOD

Participants

The data were collected as part of the Orygen Adolescent Development Study (ADS), a longitudinal research project that has been described in detail elsewhere.³⁰ Briefly, the ADS investigated biological, psychological, and social risk factors for psychopathology from 12 to 19 years of age in a community sample of participants selected from schools in the metropolitan areas of Melbourne,

Australia, to be at varying levels of temperamental risk for psychopathology. Originally, 2,479 participants were screened using the Early Adolescent Temperament Questionnaire-Revised (EATQ-R).³¹ Of these participants, 415 were selected based on their temperament scores (equal numbers across the following ranges of scores on each of the 4 higher-order factors of the EATQ-R [negative affectivity, effortful control, surgency, and affiliation]: 0–1, 1–2, 2–2.5, and >2.5 SD above and below the mean), and 245 agreed to be involved in longitudinal research. Participants were excluded from entering the study if there was any evidence of current or previous depressive, substance use, or eating disorder and were excluded from neuroimaging if there was evidence of chronic illness, language or learning disabilities, or use of medicines known to affect nervous system functioning. The sample used in the connectivity analyses ($n = 128$) and the sample used in the mediation analyses ($n = 101$) did not differ from the original 245 participants who entered the study by baseline age, sex, any temperament dimension, or socioeconomic status ($p > .05$; Table 1).

The present study used mother-adolescent interaction data available from the baseline assessment (when participants were approximately 12 years old), resting-state functional magnetic resonance imaging (rs-fMRI) data from an assessment when participants were approximately 16 years old, and data on first onset of MDD from 16 years of age, and again from a final wave of the study, at 19 years of age (Figure 1 present the study timeline). Informed consent was obtained for all participants and their parent or guardian before their inclusion in the study (and at each study wave) in accordance with the human research ethics committee of The University of Melbourne (Melbourne, Australia).

Diagnostic Interviews

The Schedule for Affective Disorders and Schizophrenia for School-Aged Children: Present and Lifetime Version³² was administered at each wave of the study to assess for lifetime diagnoses of *DSM-IV* Axis I disorder. At each wave, current and past (i.e., since last interview or since birth in the case of the first wave) psychopathology was assessed. Interviews were conducted by trained researchers under the supervision of the principal investigator (N.B.A.) and a clinical psychologist who met with researchers once a week to discuss symptoms and diagnoses. Approximately 20% of interviews were double-scored by another researcher, and inter-rater reliability was calculated on all items, including symptoms and diagnoses, using the κ statistic. At all phases, κ was in the “excellent” range, which is any value higher than 0.77.³²

Adolescent–Mother Interaction

As previously described,³³ participants and their mothers participated in 2 20-minute family interactions: an event-planning interaction (EPI) followed by a problem-solving interaction. The topics of the 2 tasks were identified based on participant responses to the Pleasant Events Checklist³⁴ and the Issues Checklist,³⁵ respectively, and were video-recorded for coding purposes. The Pleasant Events Checklist is composed of activities that people enjoy doing, whereas the Issues Checklist contains topics with the potential to create adolescent–mother conflict. The Living In Family Environments (LIFE) coding system³⁶ was used to obtain a detailed analysis of mothers’ and children’s behaviors from the video of the interactions. The LIFE consists of 10 nonverbal affect codes and 27 verbal content codes. Coding of interactions used an event-based protocol in which new codes were entered each time the affect or content of one of the interactants changed. The affect and verbal content codes were used

TABLE 1 Demographic Information of Participants in the Study Sample

	Descriptive Statistics		
	Entire Sample (N = 128) ^a	MDD Mid to Late Adolescence (n = 14) ^{b,c}	No MDD (n = 87) ^b
Age—early adolescence, mean (SD)	12.47 (0.44)	12.51 (0.30)	12.50 (0.47)
Age—mid adolescence, mean (SD)	16.47 (0.53)	16.40 (0.39)	16.50 (0.57)
Age—late adolescence, mean (SD)	18.81 (0.47)	18.73 (0.38)	18.82 (0.50)
Mother’s aggressive behavior in EPI, mean (SD) ^{d,e}	0.49 (0.46)	0.68 (0.57)	0.50 (0.45)
Mother’s positive behavior in PSI, mean (SD) ^{d,e}	1.74 (0.68)	1.63 (0.79)	1.81 (0.65)
FSIQ, mean (SD) ^f	105.61 (12.63)	107.21 (14.01)	106.68 (13.10)
SES, mean (SD) ^g	58.00 (20.30)	55.59 (17.34)	59.70 (20.64)
EATQ-R negative affectivity, mean (SD) ^d	35.80 (8.94)	36.95 (9.16)	35.98 (8.57)
EATQ-R effortful control, mean (SD) ^d	69.90 (12.27)	70.49 (16.09)	69.70 (12.04)
EATQ-R surgency, mean (SD) ^d	13.39 (3.61)	14.78 (3.62)	12.96 (3.49)
EATQ-R affiliation, mean (SD) ^d	43.69 (7.31)	47.80 (7.74)	42.74 (6.87)
Males/females	65/63	6/8	48/39

Note: EATQ-R = Early Adolescent Development Questionnaire—Revised; EPI = event-planning interaction; FSIQ = Full-Scale IQ; MDD = major depressive disorder; PSI = problem-solving interaction; SES = socioeconomic status.

^aNote that there were no sex differences for any variable ($p > .05$), except EATQ-R effortful control ($p = .028$) and affiliation ($p = .026$). This sample did not differ from the original 245 participants who entered the study by baseline age, sex, any temperament dimension, or SES ($p > .05$).

^bData included in mediation analyses. This sample did not differ from the original 245 participants who entered the study by baseline age, sex, any temperament dimension, or SES ($p > .05$).

^cThere were no differences ($p > .05$) on any variable between those with MDD from mid to late adolescence ($n = 14$) and those with no lifetime MDD ($n = 87$), except for EATQ-R affiliation ($p = .014$).

^dData collected at early adolescent assessment.

^eMaternal positive behavior was normally distributed as indicated by the Shapiro-Wilk test ($p = .940$). Maternal aggressive behavior was positively skewed ($p < .001$ by Shapiro-Wilk test, skewness statistic 0.916, standard error 0.217).

^fFSIQ was assessed using a short form of the Wechsler Intelligence Scale for Children, Fourth Edition.⁴⁶

^gSES was indicated by parental occupation status, assessed based on the Australian National University Four Scale,⁴⁷ which draws from national census data (compulsory for all Australian households) and classifies occupations by skill level and occupation type.

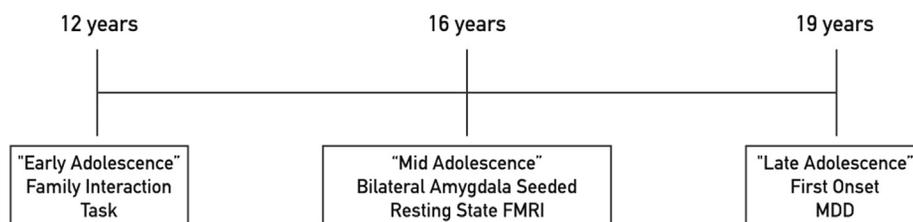
to develop composite positive and aggressive expression constructs. The positive construct included all behaviors with happy or caring affect and approving, validating, affectionate, or humorous comments made with neutral affect. The aggressive construct included all behaviors with contemptuous, angry, or belligerent affect and cruel, provocative, annoying or disruptive, or argumentative verbal statements made with neutral affect. LIFE data were used to construct a measurement of behavioral frequency for each construct, calculated as the rate per minute of a particular behavioral expression (i.e., the average number of times a mother expressed a behavior type [i.e., aggressive or positive] per minute). Although frequency of maternal aggressive behavior during the EPI was of

theoretical interest for analyses, we also investigated the frequency of maternal positive behavior during the problem-solving interaction.

Image Acquisition and Preprocessing

We used rs-fMRI data collected at 16 years of age (mean 16.46, SD 0.46) given the scant evidence on connectivity of the amygdala at this critical age when most individuals have undergone puberty but remain homogeneous with respect to school and living circumstances.^{37,38} Neuroimaging data at this time point (see below) were available for 128 participants, after excluding 13 because of head

FIGURE 1 Study timeline. Note: Adolescent participants and their mothers engaged in an event-planning task in early adolescence, resting-state functional magnetic resonance imaging (rs-fMRI) occurred in mid adolescence, and assessment of current and past major depressive disorder (MDD) occurred in late adolescence.



motion (>3 mm and or 3° rotation). The resting-state scan analyzed in this study was the first functional scan in the sequence for each adolescent.

MRI was performed with a 3-T Siemens Magnetom Trio (Siemens, Berlin, Germany) whole-body scanner equipped with 32-channel head coil at the Royal Children's Hospital (Melbourne, Australia). Resting-state fMRI (eyes closed) was performed using single-shot gradient-recalled echo-planar imaging providing T2*-weighted blood oxygenation level-dependent contrast with the following parameters: repetition time 1,400 ms, echo time 30 ms, flip angle 90°, field of view 210 mm, voxel size 3.3 × 3.3 × 5.0 mm, 24 slices, and 510 whole-brain volumes. Structural images were acquired as a gradient echo volumetric acquisition sequence (repetition time 1,900 ms, echo time 2.24 ms, field of view 230 mm) to obtain 176 T1-weighted contiguous 0.9-mm slices. A region-of-interest analysis was performed using the whole amygdala defined by the Automated Anatomical Labeling Atlas³⁹ and in which the 3 amygdala subregions (CM, LB, and SF) were defined in standard space in the right and left hemispheres as implemented in the Anatomy toolbox.⁴⁰ The 3 subregions had previously been delineated based on cytoarchitectonic probabilistic maps in histologic sections of human postmortem tissues.²⁶ The average sizes of the subregions sampled were 235 voxels (LB), 25 voxels (CM), and 107 voxels (SF; see Supplemental Method, available online, for more information). Participant sleeping during the rs-fMRI scan was assessed with a post-scan questionnaire. No participants reported falling asleep.

Image preprocessing was performed using Statistical Parametric Mapping (SPM8; <http://www.fil.ion.ucl.ac.uk/spm/>) software and included motion correction by affine transformation to the first image and co-registration of functional images with participants' anatomic scans, which were concurrently normalized to the SPM-T1 template. The resulting transformation matrix was applied to the functional data to achieve accurate spatial normalization across individuals. The anatomic scans were segmented using a unified normalization and segmentation approach.⁴¹ The SPM intensity-based segmentation algorithm thresholding values for cerebrospinal fluid (CSF) and white matter (WM) were selected to create minimal overlap between the different segmented tissue types while ensuring that gray matter tissues are not overly penalized. WM and CSF segments were generated by thresholding the corresponding tissue images segmented from the T1 scan at 99% and 50% tissue probability, respectively. Because of the high intensity of WM compared with gray matter and CSF, the threshold was more stringent so that only voxels with a high probability of containing WM were retained for subsequent analysis. CSF was hardly detected at the same stringent threshold; hence, the threshold was lowered to ensure adequate voxel coverage. The resultant combined WM and CSF mask was subtracted from the gray matter mask with a stringent threshold of 1% so that voxels containing more than 1% WM or CSF were removed. The final WM plus CSF mask was used to extract spurious signal from the resting-state data. To further control for noise and spurious signal, voxel-wise time series were extracted from the WM and CSF masks and subjected to principal component analyses using a CompCor method.⁴² The first 5 components were retained from each analysis. Then, all data were linearly detrended, and a linear regression model that included these 10 component signals and the 6 head motion parameters (3 rotation, 3 translation) estimated during the head motion correction procedure and the first-order derivatives of all 16 signals were fitted on a voxel-wise basis.⁴³ The noise-corrected data were bandpass filtered ($0.008 < f < 0.08$) and spatially smoothed with a Gaussian filter

(8 mm full-width at half-maximum). All image sequences were routinely inspected for potential normalization artifacts.

First Level, Within-Subjects Analysis

For each participant, functional connectivity maps were estimated using general linear models as implemented in SPM8. Time courses extracted from each of the amygdala seed regions were entered into a separate general linear model that included one of the amygdala seeds mean time series as a variable of interest in a whole-brain regression analysis. Contrast images were generated for each participant by estimating the positive and negative regression coefficients between all brain voxels and each region's time series for amygdala seeds.

Second Level, Within-Group Analysis

Separate second-level models were used to test for the main effect of amygdala connectivity within the 3 subregions and the whole amygdala seed. Contrast images representing positively and negatively predicted voxels for each of the 4 seeds were passed onto separate second-level *t* tests to test for connectivity in the whole brain. To investigate associations with maternal behavior, frequencies of aggressive and positive maternal expression were entered as covariates of interest in these separate models. Although there were no sex differences in amygdala connectivity analyses, sex was included as a nuisance variable. Other potential confounds (maternal depression and adolescent expression of aggression) were not significantly associated with maternal expression of aggression (see Supplementary Results, available online), and thus we did not control for these variables in analyses. Results were set at an initial voxel-wise threshold of $p < .001$ and then subjected to cluster-based family-wise error correction using Gaussian random field theory as implemented in SPM to correct for multiple comparisons. To further test the robustness of the effect, the nonparametric, threshold-free cluster enhancement method⁴⁴ was used to correct for multiple comparisons. Results survived both correction methods, so we present the results of the original analysis.

Exploratory Ventral Striatum Resting-State Connectivity Analysis

To validate the specificity of the amygdala resting-state connectivity association with maternal behavior, an additional seed-based analysis was conducted in which ventral striatum and nucleus accumbens resting-state connectivity with the rest of the brain was investigated. Notably, maternal behavior was not associated with ventral striatum connectivity (see Supplement 1 and Figure S1, available online).

Assessment of Onset of MDD and Mediation Analysis

Psychiatric data were available from 115 of the 128 participants included in the connectivity analysis. Fourteen participants (8 girls) were identified as experiencing a first incidence of MDD from 16 to 19 years of age (i.e., since the fMRI scan) compared with 101 participants without first-incidence depression during this time (see Table S1, available online, for comorbid diagnoses). Another 14 of the remaining 101 individuals had experienced a first incidence of MDD before 16 years (i.e., before the fMRI scan). To investigate whether amygdala connectivity at 16 years mediated the association between parenting and later onset of MDD (i.e., onset at 16–19 years), we conducted the mediation analyses excluding participants with MDD onset before 16 years, leaving 87 individuals with no

lifetime MDD diagnosis and 14 individuals with MDD onset after the fMRI scan. This approach ensured that rs-fMRI was not influenced by current or past MDD. Mediation analyses⁴⁵ were implemented using the Process macro in SPSS 23 (IBM Corp., Armonk, NY). A full mediation model (model 4) was tested using maternal aggression at 12 years of age as the predictor, adolescent first-onset MDD at 16 to 19 years as the outcome (i.e., excluding MDD cases before 16 years, $n = 14$), and amygdala-temporal cortex resting-state connectivity at 16 years as the mediator. Adolescent sex and comorbid non-MDD diagnoses (onset after the MRI scan) were used as covariates in the model (at the level of the outcome variable only). Nonparametric bootstrapping analysis (randomly sampled with replacement from the dataset) was used to calculate the desired statistic in each resample. The 50,000 bootstrapped resamples provide an approximation of the sampling distribution of the statistic of interest (i.e., $a*b$) with 95% CIs and is appropriate for use in smaller samples. The mediation effects were considered statistically significant if the bootstrapped CI did not include 0.

RESULTS

Descriptive data for the cohort are presented in Table 1.^{46,47} Connectivity of the whole amygdala seed represented a ventral limbic network of brain regions that was most significant in the temporal cortex, somatosensory cortex, hippocampus, striatum, thalamus, midbrain, and ventromedial prefrontal cortex (Figure 2). Negative correlation with amygdala seed region was apparent in the dorsal attention network, including the dorsolateral prefrontal cortex, parietal cortex, dorsomedial cingulate, anterior insula, and visual cortex. In general, individual amygdala subregions mirrored the connectivity of the whole amygdala seed, with the CM nucleus showing the largest pattern of connectivity across the 3 seed regions (Figure S2, available online).

Behavioral Association

Maternal expression of aggression during the EPI task showed significant positive associations with the connectivity

between the total amygdala and right Heschl gyrus, superior temporal gyrus, posterior insula, bilateral visual cortex, and left temporal and inferior insula cortices (Table 2, Figures 3 and S3, available online). Maternal aggression also showed a positive association with connectivity between the CM amygdala and the same left temporal and insula cortical regions seen for the whole amygdala (Figures 3 and S3, available online). Maternal expression of aggression was not associated with connectivity of the LB or SF nuclei and was not negatively associated with connectivity of any seed. Maternal positive behavior during the problem-solving interaction was not associated with whole amygdala or amygdala subnuclei connectivity.

Mediation Analysis

There was evidence to support mediation models for 2 amygdala connectivity results. Whole amygdala and CM amygdala connectivity with the temporal and insula cortices were associated with adolescent-onset MDD at 16 to 19 years. The association between maternal aggressive expression and late adolescent-onset MDD was mediated through whole amygdala-temporal and insula cortical connectivity (CI 0.20 to 2.87; Figure 4A) and through CM amygdala-temporal and insula cortical connectivity (CI 0.13–2.43; Figure 4B).

DISCUSSION

Consistent with our hypotheses, we observed that early adolescent maternal expression of aggression in the context of a positive interaction was associated with mid adolescent amygdala seeded resting-state functional connectivity with the temporal and insula cortices (in addition to the visual cortex). Importantly, maternal expression of aggression was positively associated with connectivity in the left temporal cortex and insula (which was driven by the CM amygdala), and these specific connectivity patterns mediated the

FIGURE 2 Z-score map of significant clusters representing positive correlation (top row) and negative correlation (bottom row) with the whole amygdala seed. *Note:* Results are cluster corrected for multiple comparisons ($p < .05$). Numbers represent coordinates in Montreal Neurological Institute standard space, with the right hemisphere displayed on the right of the image. See online version of article for full color figure.

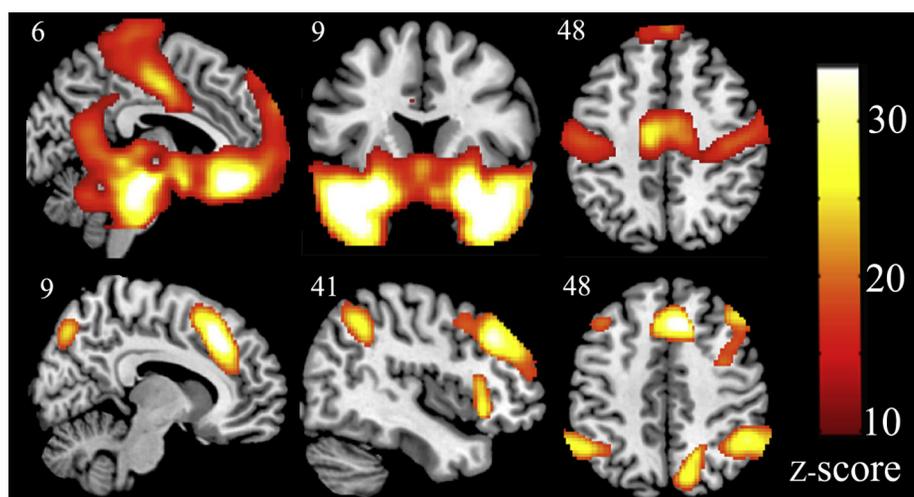


TABLE 2 Brain Regions Demonstrating Significant Association Between Amygdala Resting State Connectivity and Mother's Aggressive Behavior on the Event-Planning Interaction Task

Cluster Number	Anatomic Region	Hemisphere	MNI Peak Coordinates (x, y, z)	Z-Score	Voxels
1	posterior insula, superior temporal and Heschl gyri	right	42, -26, 4	5.07	909
2	cerebellum and visual cortex	bilateral	8, -44, -4	4.93	6,983
3	temporal and insula cortex	left	-38, 2, -18	4.20	167

Note: See Figures 3 and S3 (available online) for more information. MNI = Montreal Neurological Institute.

relation between maternal aggressive behavior and subsequent onset of case-level MDD in late adolescence. This result demonstrates the importance of maternal aggressive behavior during mother-adolescent interactions in the development of resting-state networks and how these are associated with later adolescent depression.

Although no previous literature has investigated links between maternal expression of aggressive affect and adolescent amygdala connectivity, the present data are consistent with prior literature examining the role of the parent-child relationship in shaping amygdala-based resting-state networks. For example, Rifkin-Graboi *et al.*²⁵ demonstrated that infant resting connectivity between the amygdala and temporal cortex was negatively related to maternal sensitivity in infants. In another infant study, maternal depression was associated with increased connectivity between the amygdala and temporal cortex and between the amygdala and insula.²⁴ Activity in the amygdala, insula, and temporal cortex has been related to socio-emotional processing and memory, functions that exhibit extended developmental trajectories throughout adolescence and that are disrupted in depressive disorders.^{15,48,49} Functional abnormalities in amygdala connectivity to the insula and temporal cortex also are directly related to depressive symptoms in developing individuals,^{44,49} suggesting that the effect of parenting on this circuitry might be an important pathway for the emergence of depression in adolescence.

The mechanisms by which maternal aggression during early adolescence contributes to amygdala-temporal and insula resting-state connectivity in mid adolescence are unknown, but such resting connections could reflect a history of stimulus-elicited connectivity between these regions. Recent studies have demonstrated that stable resting networks in children and adolescents can be predicted by task-based coactivations measured 2 years previously.¹⁷ Interestingly, greater amygdala-temporal cortex connectivity is observed during the encoding of emotionally arousing information⁵⁰ and is positively related to successful retrieval of emotional memory items.^{51,52} Also, amygdala-insula connectivity is increased in an emotional faces task and has been positively associated with anxiety outcomes. Whether the higher arousal presumably present in households with aggressive parents might result in greater amygdala-temporal cortex and amygdala-insula coactivation before the stabilization of this resting-state network and the onset of depression is a question for future research.

Of all the amygdala subdivisions, the widest resting-state connectivity network involved the CM amygdala seed (Figure S3, available online). This is despite the CM group being the smallest of the 3 amygdala subdivisions.²⁶ The results could indicate that the CM amygdala plays a more comprehensive role during this critically important age than previously appreciated. This notion is supported by the finding that only connectivity of the CM subdivision and

FIGURE 3 Association between amygdala connectivity and maternal aggressive behavior (see Table 1 for more information).

Note: The red circle represents clusters showing a significant association between centromedial connectivity and maternal aggressive behavior. Scatterplots are presented in Figure S3 (available online). Results are displayed in Montreal Neurological Institute standard space, with the right hemisphere shown on the right and corrected for multiple comparisons ($p < .05$). See online version of article for full color figure.

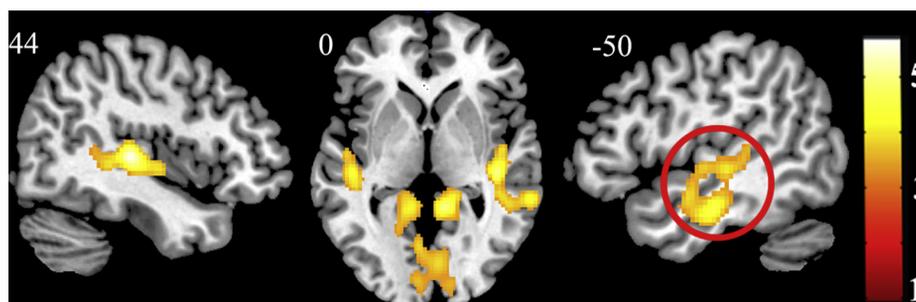
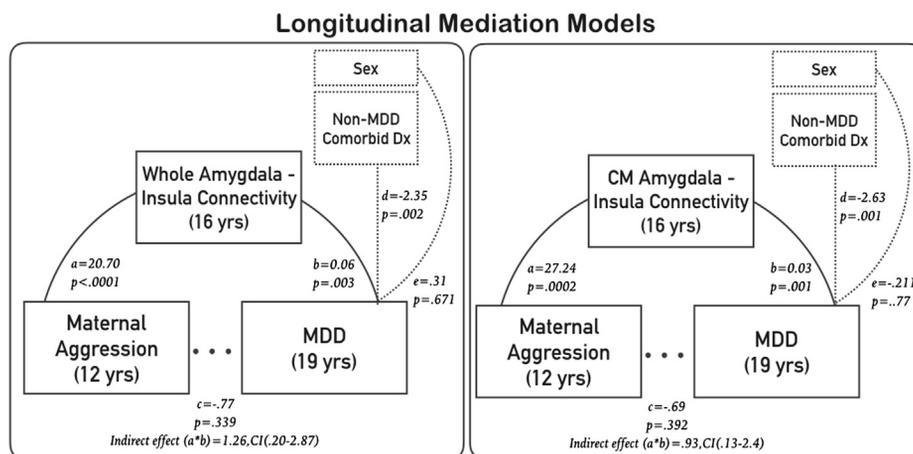


FIGURE 4 Mediation model for maternal aggressive behavior during early adolescence predicting first onset of major depressive disorder (MDD) in late adolescence, through (left) whole amygdala connectivity with the insula and temporal cortex, and (right) centromedial (CM) amygdala connectivity with the insula and temporal cortex in mid adolescence. Dx = diagnosis.



whole amygdala mediated the association between maternal expression of aggression and the onset of depressive disorder. Hence, seeding amygdala subdivisions is important to investigate the putative changes in amygdala connectivity and provides unique information on the specificity of amygdala connectivity patterns. Indeed, the central amygdala is known to be the output station for conditioned fear and anxiety through connections with numerous cortico-limbic and subcortical structures.^{53,54} Some rodent studies have implicated neuropeptide Y receptors specifically in the central amygdala as being selective for depressive behaviors,⁵⁵ and others have shown that stress-induced neurotropic changes that were specific to the central amygdala were associated with more severe depressive behaviors.⁵⁶

There are some limitations in the present design. Most significantly, the fact that paternal aggression was not investigated was a necessary (owing to low father involvement), but not theoretically supported, aspect of the study. Indeed, the father's emotional socialization of depressive-responding children might be stronger than the mother's, particularly for boys.⁵⁷ Hence, examination of paternal aggressive behavior should be a priority in future studies. In addition, rs-fMRI data were not collected at time 1 (12 years of age), precluding a calculation of change in resting-state networks as a function of maternal behavior and thus any strong inferences about directionality. However, temporal separation of the maternal aggression (12 years of age), measurement of resting-state networks (16 years), and outcomes of depression (16–19 years) in this longitudinal design are novel and superior to cross-sectional analyses. Nevertheless, future longitudinal studies with MRI data collected at multiple time points will be required to fully understand the directional nature of this relation. The amygdala subregion analysis is necessarily limited by the sizes of regions of interest than the whole amygdala approach, which can limit the interpretation of the findings. However, all the findings that were reported in the whole amygdala were then

localized to the CM amygdala. Hence, spurious findings due to low subregion coverage are highly unlikely.

There are several important strengths to the present study that warrant mentioning. In particular, the study uses a large sample and a longitudinal design, which enhances the strength with which directional attributions can be made (although we note that we did not have repeat measures of observed maternal behavior or amygdala connectivity, which prevents any causal interpretations). Further, the observational analysis of maternal aggressive expression circumvents insight and social desirability effects of self-reported maternal aggression.

This is the first study to examine the link among maternal aggressive expression in adolescence, adolescent resting-state networks, and adolescent depression. We found that whole and CM-amygdala resting networks in mid adolescence mediated the association between earlier observationally measured maternal aggression and subsequent depressive disorder diagnoses in late adolescence. These data suggest a neurobiological mechanism linking adolescent-parent interactions to depression, opening several avenues for future interventions. In particular, further research on the involvement of parents in the prevention of adolescent depression (possibly through the use of parent training) is clearly supported by these findings, and current efficacy for such approaches is good.⁵⁸ Although the measures used in this study are unlikely to be used in clinical settings (because of the time and expense of coding), knowledge about the parenting practices linked to adolescent depression (as a result of research using LIFE coding) has already been used in the development of parenting programs for adolescent mental health problems.⁵⁹ In addition, mechanistic investigations of the link between connectivity measures and psychological processes socialized by maternal aggression that might increase depression risk (e.g., negatively biased memory retrieval) should be investigated. The large burden and persistence of adolescent depression⁵⁸ and ease of accessibility

to parents as therapeutic tools support continued examination of parenting during adolescence on the development of emotion neurobiology and emotion dysregulation. &

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SUPPLEMENT 1 METHOD

Amygdala Nuclei

The sizes of the nuclei seeds varied according to their natural and cytoarchitectonic features as outlined previously^{1,2} and were the laterobasal (LB) subdivision (left, 1,840 mm³, 230 voxels; right, 1,920 mm³, 240 voxels), the centromedial (CM) subdivision (left, 176 mm³, 22 voxels; right, 224 mm³, 28 voxels), and the superficial (SF) subdivision (left, 952 mm³, 119 voxels; right, 760 mm³, 95 voxels).

Consideration of Nuisance Variables

To ensure that effects of maternal expression of aggression on amygdala connectivity (and in turn adolescent major depressive disorder [MDD]) were in fact due to maternal aggression, the potential confounding effects of maternal depression and adolescent expression of aggression were explored. Maternal history of depressive disorder during or before the early adolescent assessment was established using the Structured Clinical Interview for *DSM-IV* (SCID-IV). These data were available for 104 (of the 128) participants. Of these, 23 mothers had a history of MDD. Those with a positive history did not differ from those who did not on the measure of maternal expression of aggression ($t_{102} = 0.529$, $p = .598$) or on the measure of maternal expression of positive behavior ($t_{102} = 0.211$, $p = .833$). Adolescent expression of aggression was calculated in the same way as described for mothers. Adolescent and maternal expressions of aggression were not significantly correlated ($r = -0.121$, $p = .179$). Given that maternal depression and adolescent expression of aggression were not significantly associated with maternal expression of aggression toward adolescents,

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we did not control for these variables in any of the analyses of interest.

Exploratory Ventral Striatum Resting-State Connectivity Analysis

To validate the specificity of the amygdala resting-state connectivity association with maternal behavior, an additional seed-based analysis was conducted in which ventral striatum (VS) and nucleus accumbens resting-state connectivity with the rest of the brain was investigated. The VS is a brain region well known for its role in affect and emotional control and, hence, serves as an adequate control to further validate the specificity of the amygdala findings. An established procedure was used to characterize VS functional connectivity³⁻⁵ in which the VS seed was defined in the left hemispheres as a 3.5-mm radial sphere at the following stereotaxic coordinates ($x = -9$, $y = 9$, $z = -8$).³⁻⁵ First- and second-level analyses and associations with maternal behavior (frequency of aggressive and positive maternal expression) followed the description for the amygdala analysis.

RESULTS

Ventral Striatum

In the entire sample, VS connectivity represented a similar pattern of connectivity to the ventromedial prefrontal cortex, cingulate cortex, hippocampus, striatum, thalamus, and midbrain (Figure S1). Neither maternal expression of aggression nor positive behavior was associated with connectivity of the VS.

FIGURE S1 Z-score statistical map of ventral striatum and nucleus accumbens resting-state connectivity (left) and anti-correlation (right) with the brain. *Note:* The left hemisphere is on the left side of the image. Results are cluster corrected for multiple comparisons ($p < .05$) and presented in Montreal Neurological Institute standard space ($x = 2, y = 12, z = 51$; $x = -39, y = 36, z = 46$, respectively).

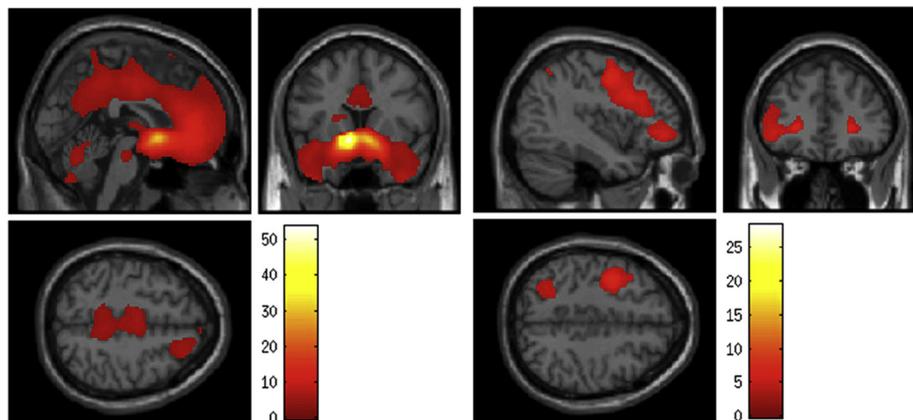


FIGURE S2 Functional connectivity of the 3 amygdala subregions: centromedial (CM), laterobasal (LB), and superficial (SF). *Note:* Significant positive (red) and negative (blue) correlations are shown in the sagittal ($x = 8$) and axial ($z = 51$) planes in Montreal Neurological Institute standard space, with the right hemisphere displayed on the right. Results are cluster corrected for multiple comparisons ($p < .05$).

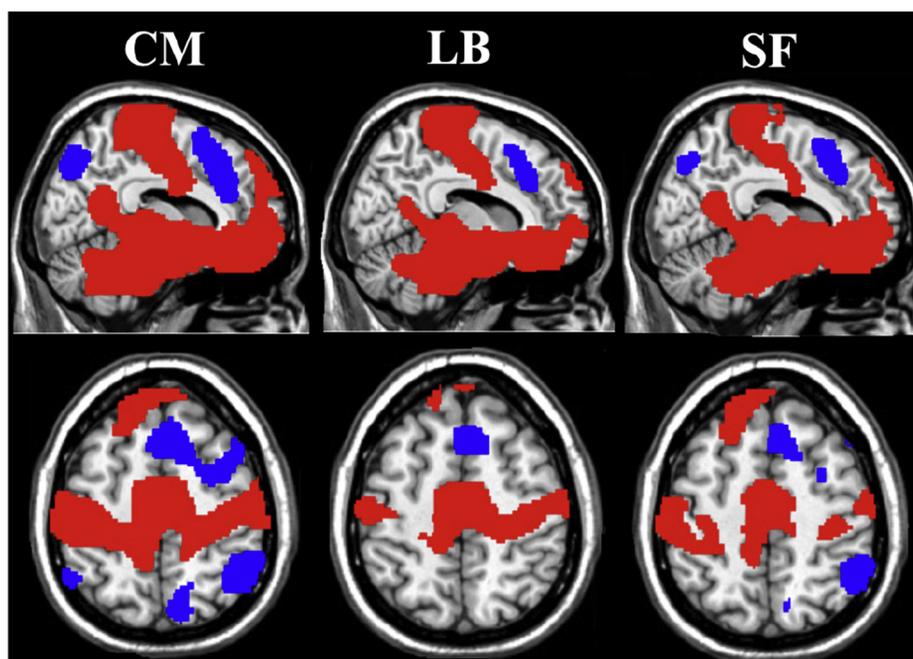


FIGURE S3 Scatterplot of the association between maternal aggressive behavior and amygdala connectivity with clusters 1 to 3. *Note:* Results are corrected for multiple comparisons ($p < .05$). See Figure 3 and Table 1 for more information. CM = centromedial amygdala.

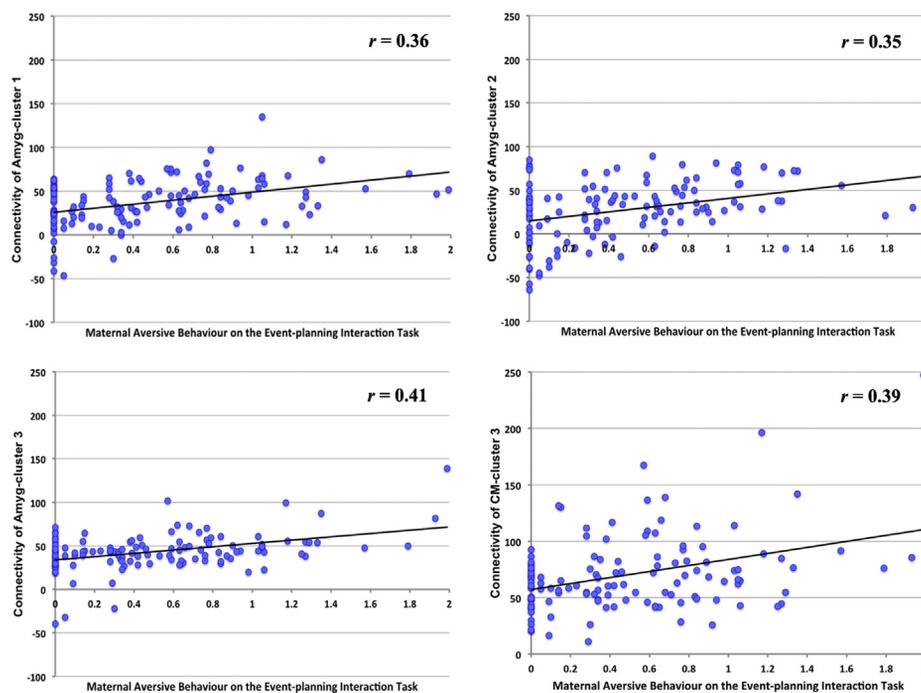


TABLE S1 Comorbid Diagnoses During Follow-Up Period From Approximately 16 to 19 Years of Age

Diagnosis	MDD Mid to Late Adolescence (n = 14)	No MDD (n = 87)
Adjustment disorder with depressed mood		1
Adjustment disorder with depressed mood, eating disorder NOS		1
Alcohol abuse		3
Bipolar I, panic disorder		1
Cannabis dependence		2
Alcohol abuse, cannabis abuse	1	
PTSD	1	
Social phobia	1	
Social phobia, GAD	1	
Specific phobia, cannabis dependence	1	

Note: GAD = generalized anxiety disorder; MDD = major depressive disorder; NOS = not otherwise specified; PTSD = posttraumatic stress disorder.