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Abnormal Amygdala Resting-State Functional Connectivity in Adolescent Depression

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Abstract

Importance—Major depressive disorder (MDD) frequently emerges during adolescence and can lead to persistent illness, disability and suicide. The maturational changes that take place in the brain during adolescence underscore the importance of examining neurobiological mechanisms during this time period of early illness. However, neural mechanisms of depression in adolescents have been understudied. Prior research has implicated the amygdala in emotion processing in mood disorders, and adult depression studies have suggested amygdala-frontal connectivity deficits. Resting-state functional magnetic resonance imaging (rsfMRI) is an advanced tool that can be used to probe neural networks and identify brain-behavior relationships.

Objective—To examine amygdala resting-state functional connectivity (RSFC) in adolescents with and without MDD using rsfMRI, and to examine how amygdala RSFC relates to a broad range of symptom dimensions.

Design—Cross-sectional rsfMRI study.

Setting—Depression research program at an academic medical center.

Participants—41 girls and boys aged 12–19 years with MDD and 29 healthy adolescents (frequency matched on age and sex) with no psychiatric diagnoses.

Main Outcome Measure—Using a whole-brain functional connectivity approach, we examined correlation of spontaneous fluctuation of blood-oxygen-level-dependent (BOLD) signal of each voxel in the whole brain with that of the amygdala.

Results—Adolescents with MDD showed lower positive RSFC between amygdala and hippocampus, parahippocampus and brain stem; this connectivity was inversely correlated with general depression, dysphoria, and lassitude, and positively correlated with well-being. Patients also showed greater (positive) amygdala-precuneus RSFC (in contrast to negative amygdala-precuneus RSFC in controls.)

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Conclusion—Impaired amygdala-hippocampal/brainstem and amygdala-precuneus RSFC has not previously been highlighted in depression and may be unique to adolescent MDD. These circuits are important for different aspects of memory and self-processing, and of modulation of physiological responses to emotion. The findings suggest potential mechanisms underlying both mood and vegetative symptomatology, potentially via impaired processing of memories and visceral signals that spontaneously arise during rest, contributing to the persistent symptoms experienced by adolescents with depression.

Introduction

Major depressive disorder (MDD) is a leading cause of disability and global disease burden[1] and frequently emerges during adolescence.[2] Many adolescents do not respond to evidence-based treatments,[3, 4] highlighting the need to better understand the pathophysiology. Current theory holds that fronto-limbic neural networks underlying emotion processing are abnormal in MDD.[5, 6] However, neurobiological research in adolescents has lagged behind that of adults. Due to the significant brain maturational changes that occur during adolescence,[7] pathophysiology in adolescent MDD could be different than in adults. Developmental changes may contribute to the increased risk of disease onset during adolescence, while also providing a potential window for intervention to restore developmental trajectories. These considerations underscore the importance of advancing understanding of the neurobiology of adolescent MDD.

The amygdala, an important area for processing threat and orchestrating a complex set of emotional and physiological responses,[8] has been centrally implicated in depression.[9] Amygdala networks are involved in critical functions relevant to depression including emotion regulation (through connections to frontal and insular areas), modulation of sensory information (through connections with visual, auditory, taste and olfactory cortices), and processing of visceral information in relation to emotional stimuli (through connections with the brain stem).[10] Based on the importance of amygdala in emotion systems and its implication in MDD, the current study is focused on examining amygdala networks in adolescents with MDD.

Resting-state functional magnetic resonance imaging (rsfMRI) is an excellent tool for probing neural networks. This approach measures resting-state functional connectivity (RSFC) indexed by the correlation between brain regions in the pattern of spontaneous fluctuation of blood oxygen level dependent (BOLD) signal during rest.[11] Positive and negative correlations are understood to reflect synchrony in regions subserving similar and opposite goals, respectively.[12] Prior studies have shown that rsfMRI can reliably map RSFC in adults[13] and children.[14] Research in adults has suggested that MDD involves a deficit in amygdala-frontal connectivity.[15] In the first-published rsfMRI study on adolescent depression, we failed to find amygdala RSFC abnormalities in 12 (mostly medicated) adolescents with MDD compared to healthy 14 controls (HC), but documented abnormally low RSFC in a subgenual anterior cingulate cortex (ACC)-based network.[16] Since then, several studies have reported abnormal RSFC in children or adolescents with MDD.[17–21] However, the only study focusing on amygdala reported that children at risk for depression (due to personal and/or maternal history) had lower negative amygdala RSFC than HC with

a dorsal cognitive control networks, and lower positive RSFC with an inferior limbic network.[20]

The primary goal of this study was to examine amygdala RSFC in adolescents with MDD and HC. To extend beyond prior work, we examined a larger sample of unmedicated adolescents with fully-syndromal MDD and no substance-abuse disorders. Taking into account recent concerns in rsfMRI research,[22–24] we incorporated robust methods to address physiological noise and subject motion during scanning, without global signal removal. We predicted that, like adults with MDD,[15] adolescents with MDD would show diminished amygdala-frontal RSFC. Given the continuing development of amygdala-frontal projections into adulthood[25] and sexual dimorphism in adolescent brain development (e.g.[26]), we explored group-by-sex and group-by-age interactions. Finally, we explored how amygdala RSFC related to overall depression severity as well as broad set of depression and anxiety symptom dimensions.

Methods

Participants

The University of Minnesota Institutional Review Board approved this study. Participants (or a parent if under 18) provided written informed consent. Participants aged 17 years and younger provided written assent. Adolescents with MDD and HC aged 12 to 19 years were recruited to participate through community postings and referrals from local mental health services. MDD participants were eligible if they had a primary diagnosis of MDD and had not received any psychotropic medication treatment for the past 2 months. HC participants were eligible if they had no current or past psychiatric diagnoses, and were frequency matched to the MDD group on age and sex. Exclusion criteria for both groups included the presence of a neurological or chronic medical condition, mental retardation, pervasive developmental disorder, substance use disorder, bipolar disorder, or schizophrenia.

Assessment

After the informed consent process, all participants completed a comprehensive diagnostic assessment. Interviews were conducted separately with adolescents and parents, and included Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version[27] and the Children's Depression Rating Scales—Revised (CDRS-R).[28] Self-report measures assessing symptoms in the past two weeks included the Beck Depression Inventory II (BDI-II)[29, 30] and the Inventory of Depression and Anxiety Symptoms (IDAS).[31–33] The IDAS provides a score for the following symptom dimensions: general depression, dysphoria, lassitude, insomnia, suicidality, appetite loss, appetite gain, ill temper, well-being, social anxiety, panic, and traumatic intrusion.

MRI Data Acquisition

Data were acquired at the Center for Magnetic Resonance Research at UMN using a Siemens 3T TIM Trio scanner. A five-minute structural scan was acquired using a T1-weighted high-resolution magnetization prepared gradient echo (MPRAGE) sequence: TR = 2530ms; TE = 3.65ms; TI = 1100ms; flip angle = 7 degrees; 1mm slices, FOV = 256, voxel

size 1×1×1mm; GRAPPA = 2. The six-minute rsfMRI scan (30 minutes into the overall protocol) was comprised of 180 contiguous echo planar imaging (EPI) whole brain volumes with TR = 2000ms; FOV = 256; voxel size 3.43×3.43×4mm; 34 slices; 64×64 matrix, during which participants were instructed to stay awake with their eyes closed. Physiological data (respiration and cardiac traces) were simultaneously collected. A field map was collected.

Anatomical Imaging Preprocessing

FreeSurfer Version 5.3 (surfer.nmr.mgh.harvard.edu) was used to process T1 data including brain extraction and parcellation of data into a standard set of anatomically-based regions of white and grey matter. FreeSurfer output was visually inspected; when any errors were identified ($n = 2$) they were manually corrected on a slice-by-slice basis. After ensuring the corrections were satisfactory, the pipeline's remaining steps were repeated. No corrections were required in the vicinity of the amygdala. The processed T1 data was registered to the rsfMRI data using *bbregister*.

Resting-State fMRI Preprocessing

Image processing was conducted using tools from the FMRIB software library (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) as well as custom tools developed in MATLAB. Initial processing included brain extraction and motion correction. A denoising procedure was applied incorporating RETROICOR[34] to remove physiological noise caused by cardiac and respiratory cycles as well as any linear trends. Correction for magnetic field inhomogeneity-induced geometric distortion was conducted using the field map. FreeSurfer-generated regions of interest (ROIs) for lateral ventricles (cerebrospinal fluid; CSF) and white matter (WM) were aligned to rsfMRI data using FLIRT. Mean BOLD time series within these ROIs were extracted using *fslmeants*. We performed a regression of each other voxel's time series on eight nuisance variables: WM time series, CSF time series, and the six motion parameters. Data scrubbing was performed following Power and colleagues,[22] excluding any volume with a value for the temporal derivative of time courses' root mean squared head motion variance (DVARS value) exceeding 8 and/or a framewise dependent value exceeding 0.5, along with the previous volume and the two following volumes. If at least 33% of volumes were removed, participants were excluded from analyses (two MDD and three HC).

RSFC Analysis

First-level—A seed-based, whole-brain approach was used to examine RSFC stemming from left and right amygdala. To avoid misregistration errors, we used anatomically-based ROIs. FreeSurfer-based right and left amygdala ROIs were registered to the pre-processed rsfMRI data, and average time series of voxels in these regions were extracted. These time series were used as primary regressors in separate (left and right) general linear model analyses of each other voxel's time series, resulting in whole-brain amygdala RSFC maps. We used Gaussian Random Field Theory to correct for multiple testing using a cluster threshold of $p < 0.05$ and $z > 2.3$. Additional processing steps included spatial smoothing (5mm kernel), prewhitening, and registration to anatomical data and standard space (MNI 152)[35] for later group analysis.

Second-level—As noted in previous work,[20] amygdala RSFC maps for left and right amygdala were highly similar to each other (see eFigures 1–2). Therefore, following others,[20] to limit the number of tests and for ease of presentation, we conducted a second-level analysis to average each person's right with their left amygdala RSFC maps.

Third-level—To address the primary study question, we conducted a voxel-wise analysis of mean amygdala RSFC comparing groups, including covariates of age and sex using Gaussian Random Field Theory to correct for multiple comparisons, with a cluster z threshold of 2.3 and $p < 0.05$. We also conducted exploratory analyses to examine group-by-sex and group-by-age interaction effects on amgydala RSFC throughout the brain.

Fourth-level—A series of follow-up analyses used the significant clusters resulting from group analyses as a mask to extract average z-scores from each participant's un-thresholded amygdala RSFC map. Within the MDD group, Pearson correlations were conducted on these z-scores with symptom severity (CDRS-R and BDI-II total scores) and IDAS symptom domains. To account for multiple analyses conducted, used Holm's stepdown Bonferroni Approach (Holm, 1979). Holm's procedure is less conservative than Bonferroni and, similar to Bonferroni, does not require that the tests be independent. To explore whether additional clinical factors such as prior medication exposure or presence of a comorbid anxiety disorder might have influenced the results, we also compared mean amygdala RSFC within these clusters between adolescents with MDD who were and were not medication naïve, and between adolescents with MDD with and without a current anxiety disorder using an independent samples *t* test.

Results

Participants

Forty-three unmedicated adolescents with MDD and 31 HC participants completed all procedures. After excluding participants with excessive motion, 41 MDD (73% medication-naïve) and 29 HC adolescents were included in our final analyses (Table 1). There were no significant differences between groups with respect to age, sex or handedness. As expected, the groups differed significantly with respect to CDRS-R scores, BDI-II scores, and IDAS dimension scores. No group differences were detected between MDD medication-naive and medication-free participants, with the exception of IDAS scores for insomnia, panic, and social anxiety (see eTable 1). In the final sample, the number of excluded volumes was marginally different between groups ($U(69) = 440.5$, $p = 0.053$), largely because HC had fewer people with zero excluded volumes (see eFigure 3).

Group Differences in Amygdala RSFC

Adolescents with MDD showed lower positive amygdala RSFC than HC with a cluster that included left hippocampus and parahippocampus, a small piece of orbitofrontal cortex and temporal pole, and also extended into the brain stem (see Figure 1 and Table 2. Also see eFigure 4 for depiction of the orbitofrontal involvement). Additionally, MDD and HC adolescents differed in amygdala RSFC with bilateral precuneus, where patients showed positive RSFC, while controls showed negative RSFC (see Figure 2 and Table 2). Follow-up

analyses revealed no significant group differences between MDD medication-naïve and medication-free participants in these circuits, or between MDD participants with (*n*=25) versus without (*n*=16) a comorbid anxiety disorder (defined by the presence of any current anxiety disorder). Our whole-brain analyses to examine group-by age interaction and group-by-sex interaction in mean amygdala RSFC did not reveal any significant clusters. Additionally, when the specific regions that showed group differences were further examined (precuneus and left hippocampus/parahippocampus/brain stem), there were no significant group-by-age or group-by sex interactions.

Correlations with Symptom Domains

Within the depressed group, we used Pearson correlations to examine how amygdala RSFC scores within the clusters identified above relate to clinical severity and IDAS dimensions (Table 3). Several significant correlations were noted for the amygdala-hippocampal/brainstem circuit, where participants with lower positive RSFC in this circuit had greater IDAS general depression, dysphoria and lassitude scores, and lower IDAS well-being scores. However, the summary scores on the CDRS-R and the BDI-II were not significantly correlated with RSFC in identified amygdala networks.

Discussion

In this study we report abnormal amygdala RSFC in adolescents with MDD compared to HC. The pattern of findings has not been previously identified in the depression literature and may represent important new information about the pathophysiology of MDD in adolescents. Strengths of the present study include the relatively large sample of un-medicated adolescents with MDD (approximately twice the sample size of recent rsfMRI papers in similar populations)[17, 18] and the rigorous methods used to remove noise due to physiological signals and motion. Additionally, the results of this study identify a fruitful avenue of future work by providing preliminary evidence that abnormal circuits map on to specific symptom dimensions.

Amygdala-Hippocampus/Parahippocamus RSFC

In this study, adolescents with MDD showed lower positive RSFC than HC between amygdala and a cluster involving left hippocampus and parahippocampus, and this abnormality was associated with lower sense of well-being and higher levels of general depression, dysphoria and lassitude. The amygdala is known to be richly connected with the hippocampus and parahippocampus,[36, 37] and positive RSFC between amygdala hippocampus/parahippocampus has been shown in healthy adults.[38] Animal models suggest that amygdala-hippocampal connections facilitate the modulation of emotional memories,[39–41] and prior work using task fMRI in healthy adults has shown that amygdala-hippocampal connectivity increases during encoding and retrieval of emotional memories.[42–44] A study in adults with MDD using a memory task found that patients showed *greater* amygdala-hippocampal connectivity than controls during successful encoding of negative emotional memories, but no group differences were found for neutral or positive memories.[45] However, similar to our findings, a recent study of adults with MDD that used a whole brain, multivariate pattern classification approach identified

amygdala-hippocampus as one of many connections showing *lower* RSFC than HC,[46] and two reports in populations at risk for MDD showed similar findings.[20, 47] Therefore, it could be that in patients with or at risk for depression, the circuit is under-connected during rest, potentially as a compensatory process to off-set the hyper-connectivity that may occur during processing of negative emotional memories, and/or the general hyperactivity of amygdala in depression.[48, 49] These speculations require further investigation examining (a) the dynamic change of amygdala-hippocampal connections across states of rest, memory encoding and memory retrieval; (b) whether this abnormality represents a direct manifestation of illness or an adaptation due to another abnormality (e.g. excessive amygdala activation in depression); and (c) how RSFC and the related functions of this circuit might be restored as a consequence of treatment for depression.

Amygdala-Brainstem RSFC

Our findings show decreased RSFC between amygdala and brainstem in adolescents with MDD, which to our knowledge has not previously been reported in depression literature. Animal research has identified amygdala-brainstem connectivity as an important network for modulating visceral function in relation to emotional stimuli.[10] Excitatory pathways extend from amygdala to brainstem centers such as periaqueductal gray, locus ceruleus, raphe nucleus, and autonomic-related brainstem nuclei; modulatory pathways from these centers project back to the amygdala.[50] These pathways are important for basic functioning such as arousal and appetitive drives. In the current study, RSFC in this circuit correlated with lassitude, and, at a trend level of $p \leq 0.05$, appetite loss and insomnia. These preliminary findings suggest that impaired connectivity in this circuit underlies some of the vegetative aspects of depression. Further research probing this hypothesis with experimental paradigms to assess arousal systems are needed to test this hypothesis.

Amygdala-Precuneus RSFC

In this study, adolescents with MDD had positive RSFC between amygdala and precuneus in contrast to healthy adolescents who showed negative RSFC in this circuit. The precuneus is involved in processing of self-relevant information[51–57] and in episodic memory encoding and retrieval.[55, 58] It is an important node within the default mode network, a group of brain regions that are more active at rest than during a task.[59] Negative amygdala-precuneus RSFC has been documented in studies of healthy adults[38, 60]. Again, although this circuit has not previously been highlighted in depression literature, recent reports have noted a similar pattern in adults with high levels of neuroticism had positive amygdala-precuneus RSFC,[61] children with personal or maternal history of MDD,[20] and adults with a history of childhood maltreatment.[47] Together, these findings suggest that impaired negative RSFC (or in the case of our study, the presence of positive connectivity) between two regions with opposing functions (rest versus threat) may be an important mechanism in depression. Positive synchrony between these regions during rest could underlie a failure to suppress negative self-thoughts that spontaneously emerge during rest. Alternatively, this synchrony could contribute to “disproportionate emotional coloring of self-referential or autobiographical information processing.”[61] Both of these possibilities could feasibly perpetuate clinical features seen in depression such as rumination and the persistently negative mood state.

Amygdala-Frontal RSFC

We predicted that adolescents with MDD would show an amygdala-frontal RSFC deficit. However, the results revealed the deficit to be primarily in subcortical regions (e.g. hippocampus and brain stem). Only a small piece of the cluster representing lower amygdala RSFC in patients than controls extended into orbitofrontal cortex (eFigure 4). Several prior studies in adults have documented impaired amygdala-frontal RSFC, with mixed results regarding location and whether the impairment is in positive or negative RSFC.[15, 62–64] Variance in findings across depression studies could arise from methodology differences, heterogeneity of illness, and/or developmental effects.[65] Perhaps because the frontal lobe and its limbic connections are still developing during adolescence,[7, 25] adolescents with depression show a different pattern than adults, with more prominent findings in subcortical areas that mature earlier. It may be that amygdala-frontal RSFC deficits emerge during early adulthood as the MDD versus HC gap in frontal development trajectories widen. Although the results of our age-by-group interaction analysis do not support this hypothesis, longitudinal research examining the trajectory of amygdala RSFC across development into adulthood in youth with and without MDD will be necessary to further examine this question.

Limitations

We have interpreted our amygdala RSFC group difference findings based on the clinical features of depression and what is known about the function of the implicated brain regions. However, these interpretations of our observational data should be considered preliminary and speculative. Confirmation of the hypotheses suggested here will require further research utilizing a multi-modal approach that includes behavioral methods capable of investigating the function of the circuits in question (e.g. self-processing, emotional memory, etc.). Further, our findings regarding clinical correlations between RSFC and symptom dimensions should be interpreted with caution because large number of tests that were conducted relative to the sample size. Future research is needed with larger samples to further examine the relationships with symptom dimensions.

The cross-sectional nature of this study prohibits causal interpretations of the results. It is unclear whether the abnormalities reported represent risk markers for MDD or if they emerge during the course of illness as a result of disease processes. Longitudinal research using these measures, ideally beginning with high-risk adolescents prior to illness onset and tracking the course of illness after onset, is needed to address these questions.

Similar to other adolescent depression studies (e.g. [16–18, 66]) the participants in our sample had relatively high rates of current comorbid anxiety disorders. This is a limitation because the findings may not be specific depression. However, post-hoc analyses comparing patients with and without anxiety disorders on the amygdala-hippocampus and amygdala-precuneus circuits did not reveal any significant differences. Further, because there were no significant associations between the main amygdala connectivity findings with any of the anxiety dimensions from the IDAS, the abnormalities appear to be more related to depression than anxiety symptoms in these patients.

As has been recently highlighted in the literature,[22, 23] subject motion during the scan can significantly impact rsfMRI findings. Several methods have been proposed outlining approaches to reduce the impact of motion artifacts, one of which we incorporated in our study.[22] We removed volumes exceeding our threshold, resulting in variance across participants in the number of volumes for final analysis. As RSFC can change over time,[67] this introduces the possibility that removed volumes could potentially have altered the overall RSFC measure. We and others[22] believe that the potential for introducing artificial correlations from motion artifacts was a far greater risk than that of losing these short, randomly-spaced epochs of resting data.

Certain limitations arise from our seed-based approach. We used a hypothesis-driven approach for our rsfMRI data analysis, correlating the time series of a seed ROI with every voxel as an index of whole-brain functional RSFC.[11] This approach limits the results based on which seed region is chosen. Data-driven approaches avoid this limitation, but results can be more difficult to interpret. Further, we used an ROI of the entire amygdala, but prior work has shown that amygdala subregions have known dissociable functional networks.[38] Future research should investigate how RSFC patterns in adolescent MDD vary across amygdala subregions; such research would benefit from recent advances in acquisition methods which allow for higher spatial resolution.[68] Many studies define seeds by creating a sphere in standard space around a location from published literature,[16, 69] or using an atlas-defined region.[15] Such approaches have limitations inherent to between-person differences in anatomy. To address this, we defined our seed regions based on each individual's anatomy using FreeSurfer. This introduces the potential limitations of the automated approach to accurately parcellate the anatomy. However, FreeSurfer-based parcellation of the amygdala is superior in some respects compared to other automated methods.[70, 71] Several studies have been recently published using FreeSurfer to investigate amygdala volume in different populations with a range of psychopathology.[72–75] Further, we visually inspected each person's FreeSurfer results to identify and correct errors, which did not occur in our regions of interest.

In summary, we report abnormal amygdala RSFC in the largest sample to date of adolescents with MDD. The findings could reflect impairments in the networks that process spontaneous memories that arise during rest and underlie persistent negative mood and vegetative symptoms in these adolescents. Future research using multi-modal approaches that incorporate experimental paradigms to probe relevant systems implicated in memory, self-processing and arousal would be ideal for further illuminating brain-behavior relationships in adolescents with MDD. Given the differences from previous findings in adults, it may be that RSFC abnormalities evolve over the course of development. Future longitudinal research is needed to understand how RSFC changes over development, course of illness, and treatment response in adolescents with MDD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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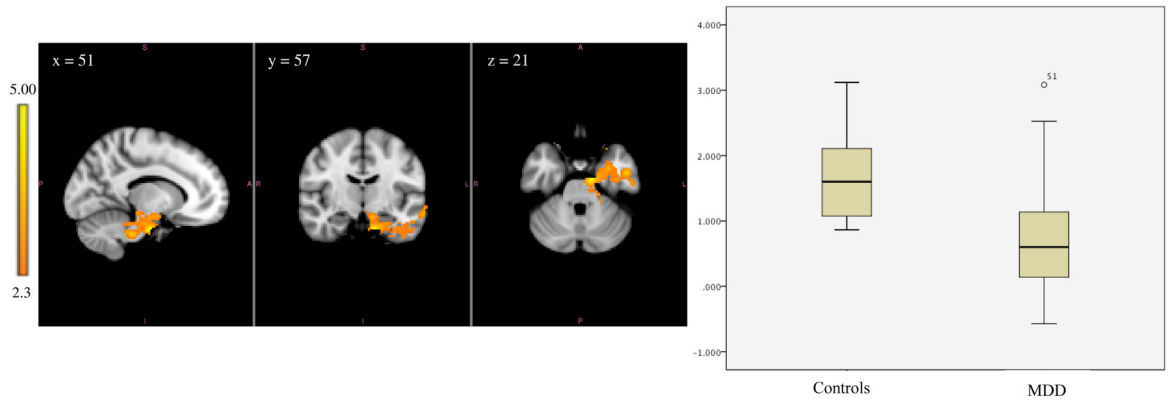

Figure 1. Lower Amygdala Connectivity in Adolescents with MDD than Controls

Left image depicts functional connectivity of amygdala-hippocampus and amygdala-brainstem in which the control group has higher functional connectivity compared to the MDD group. The coordinates represent the position of the voxel with the highest intensity in MNI standard space (z-stat = 5.00); Right image compares the range of functional connectivity z-scores between the amygdala and these regions for the two groups.

*Note: Analyses were repeated with MDD outlier removed and remained significant; $t(67) = 5.77$, $p < .001$

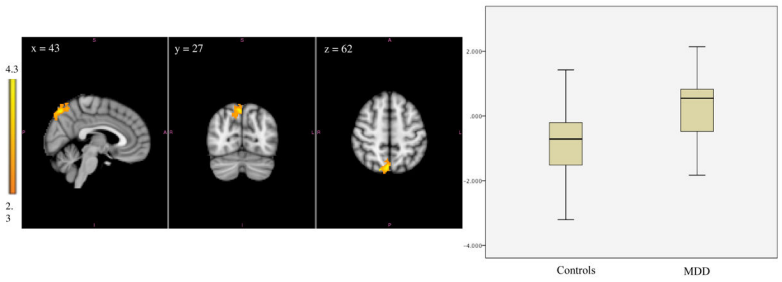

Figure 2. Greater Amygdala Connectivity in Adolescents with MDD than Controls

Left image depicts functional connectivity of amygdala-precuneus in which the MDD group has higher functional connectivity compared to the control group. The coordinates represent the position of the voxel with the highest intensity in MNI standard space (z-stat = 4.3). ; Right image compares the range of functional connectivity z-scores between amygdala to precuneus for the two groups.

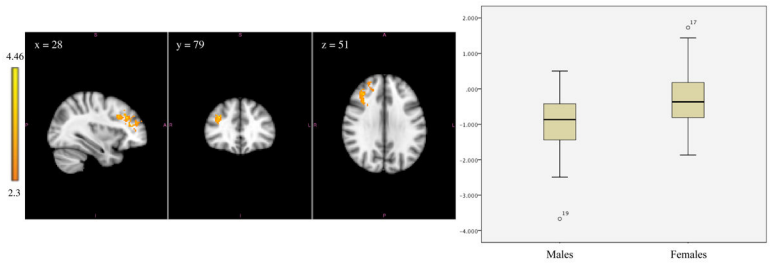

Figure 3.

Left image shows amygdala-frontal functional connectivity in which males show greater negative functional connectivity compared to females. The coordinates represent the position of the voxel with the highest intensity in MNI standard space (z-stat = 4.46); Right image compares the range of functional connectivity z-scores between the amygdala and these regions for the two groups.

Table 1

Demographic and Clinical Characteristics

Demographic Characteristics	**MDD**	**HC**	**P value**[1]
N	41	29	
Age (mean years ± SD)	15.7 ± 2	16.0 ± 2	0.5
Sex (male/female)	9/32 (78% female)	7/22 (76% female)	0.8
Right Handed – n (%)	32 (91%; n = 35)	25 (93%; n = 27)	0.8
***Ethnicity* – n (%)**			0.1
Caucasian	28 (68%)	16 (55 %)	
African American	5 (12%)	1 (4%)	
Hispanic	4 (10%)	4 (14%)	
Asian	0	2 (70%)	
Native American	0	1 (3%)	
Other	4 (10%)	5 (17%)	
Current Comorbidities – n (%)	27 (68%)	N/A	
Attention Deficit and Hyperactive Disorder	6 (15%)	N/A	
Generalized Anxiety Disorder	16 (39%)	N/A	
Obsessive-Compulsive Disorder	1 (3%)	N/A	
Oppositional Defiant Disorder	2 (5%)	N/A	
Post-Traumatic Stress Disorder	2 (5%)	N/A	
Social Anxiety	3 (8%)	N/A	
Dysthymia	2 (5%)	N/A	
Panic Disorder	2 (5%)	N/A	
Specific Phobia	3 (8%)	N/A	
Social Phobia	4 (10%)	N/A	
Medication History			
Med-Naïve – n (%)	30 (73%)	N/A	
Past Antidepressant Use –n (%)	8 (57%)	N/A	
Past Stimulants Use	4 (29%)	N/A	
Past Antipsychotic Use	2 (14%)	N/A	
Illness History, Description, Etc			
Duration of illness (mean months±SD)	10 ± 11 (n = 39) [1]	N/A	
Global Assessment of Functioning (mean±SD)	54 ± 9	N/A	
Positive Family History	28 (82%; n = 34)	N/A	
Clinical Severity			
CDRS-R (T-scores mean±SD)	77 ± 6 (n = 34)	N/A	
BDI-II Most Severe (mean±SD)	29 ± 13	3 ± 4	<0.0001
IDAS Dimension scores	n = 37	n = 28	
General Depression Score	57 ± 16	27 ± 4	<0.0001

Demographic Characteristics	**MDD**	**HC**	**P value**[1]
Dysphoria Score	28 ± 9	12 ± 3	<0.0001
Lassitude Score	19 ± 6	9 ± 3	<0.0001
Insomnia Score	5 ± 6	7 ± 1	<0.0001
Suicidality Score	13 ± 7	6 ± 0.2	<0.0001
Loss of Appetite Score	7 ± 3	3 ± 1	<0.0001
Appetite Gain Score	6 ± 4	4 ± 2	0.008
Ill Temper Score	12 ± 6	6 ± 2	<0.0001
Well-being Score	18 ± 6	27 ± 6	<0.0001
Social Anxiety Score	123 ± 5	6 ± 1	<0.0001
Panic Score	14 ± 7	8 ± 1	<0.0001
Traumatic Intrusion Score	8 ± 4	4 ± 0.5	<0.0001

[1] P values resulted from chi square analyses (sex, ethnicity) or independent samples t-tests (all others).

[2] Exact number of subjects are provided

Table 2

Size and peak z-values of the significant clusters in the group analyses

Contrast	Brain Regions	# of Voxels	MNI Coordinates of Peak Voxel (x, y, z)	Peak z-value	Cluster mean z-value ± Standard Deviation
Controls>MDD	Total cluster	1831	51, 57, 21	5.00	Controls: 1.70 ± 0.67 MDD: 0.75 ± 0.83
	Left hippocampus	143			
	Left parahippocampus	274			
	Brainstem	362			
	Left orbitofrontal cortex	46			
	Left temporal pole	34			
	Left temporal fusiform	247			
MDD> Controls	Bilateral precuneus	682	43, 27, 62	4.30	Controls: −0.79 ± 1.1 MDD: 0.26 ± .89

Table 3

Correlations between amygdala connectivity z-scores and symptom domains for the MDD group

	Hippocampus/parahippocampus/brainstem *R (p)* *	**Precuneus R (*p*)** *
Clinical Severity		
CDRS-R total	−0.180 (0.93)	−0.032 (1.00)
BDI-Total	−0.324 (0.28)	−0.101 (1.00)
IDAS Dimensions		
General Depression Score	**−0.523 (0.012)**	0.043 (1.00)
Dysphoria Score	**−0.455 (0.050)**	0.047 (1.00)
Lassitude Score	**−0.449 (0.050)**	−0.920 (1.00)
Insomnia Score	−0.321 (0.30)	0.200 (0.24)
Suicidality Score	−0.202 (0.92)	0.140 (0.41)
Loss of Appetite Score	−0.384 (0.16)	0.081 (0.64)
Appetite Gain Score	0.093 (1.0)	0.114 (0.50)
Ill Temper Score	0.114 (1.0)	−0.019 (0.91)
Well-being Score	**0.470 (0.03)**	0.179 (0.29)
Social Anxiety Score	−0.289 (0.40)	−0.046 (0.79)
Panic Score	−0.184 (1.00)	0.117 (0.49)
Traumatic Intrusions	−0.014 (1.00)	0.247 (0.14)

* *p* values are corrected for multiple comparisons using Holm's stepdown Bonferroni approach (Holm 1979). Results that have corrected $p \leq 0.05$ are shown in bold.