Using novel imaging approaches in affective disorders : beyond current models

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Chapter 5
Aberrant resting-state functional connectivity in limbic and salience networks in treatment-naïve clinically depressed adolescents

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Abstract

Depression is prevalent and typically has its onset in adolescence. Resting-state fMRI could help create a better understanding of the underlying neurobiological mechanisms during this critical period. In this study, examines resting-state functional connectivity (RSFC) is examined using seed regions-of-interest (ROIs) associated with three networks: the limbic network, the default mode network (DMN) and the salience network. 26 treatment-naïve, clinically depressed adolescents of whom 18 had comorbid anxiety, and 26 pair-wise matched healthy controls underwent resting-state fMRI. The three networks were investigated using a seed-based ROI approach with seeds in the bilateral amygdala (limbic network), bilateral dorsal anterior cingulate cortex (dACC; salience network) and bilateral posterior cingulate cortex (default mode network). Compared to healthy controls, clinically depressed adolescents showed increased RSFC of the left amygdala with right parietal cortical areas, and decreased right amygdala RSFC with left frontal cortical areas including the ACC, as well as with right occipito-parietal areas. The bilateral dACC showed decreased RSFC with the right middle frontal gyrus, frontal pole, and inferior frontal gyrus in clinically depressed adolescents. No abnormalities in DMN RSFC were found, and differences in RSFC did not correlate with clinical measures. The aberrant RSFC of the amygdala network and the dACC network may be related to altered emotion processing and regulation in depressed adolescents. Our results provide new insights into RSFC in clinically depressed adolescents and future models on adolescent depression may include abnormalities in the connectivity of salience network.

Introduction

Depression is a common mental health disorder in childhood and adolescence with a prevalence of 4–5% (Thapar *et al.*, 2012). Adolescent depression leads to social and educational impairment, and also constitutes an increased risk of obesity, smoking and substance misuse, and suicide (Thapar *et al.*, 2012). Anxiety frequently co-occurs with depression, especially in youth (Simms *et al.*, 2012) and a double diagnosis accounts for more impairment, more severe internalising symptoms (Beesdo *et al.*, 2009), and a more severe emotional disturbance (Kessler *et al.*, 2012) than either diagnosis alone. Since depression and anxiety typically have their onset in adolescence, this period may be critical in terms of the underlying neurobiological events (Blakemore, 2012).

Recent years have witnessed an increase in the number of neuroimaging studies in adolescent/juvenile depression and anxiety, with the majority of the functional MRI (fMRI) studies using task paradigms (Thomas *et al.*, 2001, Pine, 2007, Hulvershorn *et al.*, 2011). Amygdala as well as ventrolateral prefrontal cortex hyperactivation was frequently found in anxiety disorders (Pine, 2007). Similarly, altered amygdala activation was also linked to adolescent depression (Hulvershorn *et al.*, 2011). While task fMRI studies have provided important insights into altered neural processing within individual brain areas, models of depression emphasize the importance of functional connections between brain regions (Mayberg, 1997).

Resting-state fMRI allows researchers to investigate such functional connections without using externally controlled task paradigms, focusing on spontaneous fluctuations in brain activity (Biswal *et al.*, 1995, Fox and Raichle, 2007). The number of resting-state functional connectivity (RSFC) studies in juvenile depression and anxiety is small (Pine, 2007, Hulvershorn *et al.*, 2011) with one recent study in anxiety disorders (Roy *et al.*, 2013) and seven in depression. These studies typically focus on just one specific network of interest. Furthermore, the variance in medication history among subjects could be a significant confounding factor (Wang, 2012). Aberrant RSFC was reported for the subgenual anterior cingulate cortex (ACC), the limbic-striatal system, the dorsolateral, medial and inferior prefrontal cortex (PFC), and the amygdala, insula and temporal cortices. These studies have suggested aberrancies in salience attribution

and executive control related to subgenual ACC connectivity (Connolly *et al.*, 2013), in emotion processing related to subgenual ACC connectivity with frontotemporal cortical areas (Cullen *et al.*, 2009) and a range of frontal and temporal cortical areas as well as the amygdala (Jin *et al.*, 2011). Additionally, abnormalities have been suggested in cognitive emotional control, related to subgenual ACC connectivity with thalamic and parietal regions (Gaffrey *et al.*, 2010) and fronto-subcortical circuits (Jiao *et al.*, 2011), and in emotion regulation related to DMN connectivity (Gaffrey *et al.*, 2012) and the amygdala (Luking *et al.*, 2011). Despite the regularity with which the involvement of the ACC, amygdala, and medial PFC is reported in depression, inconsistency remains in terms of increased or decreased connectivity and correlations with symptom severity.

The amygdala, which is central in the fear system with an important function in detecting, signalling, and learning from threat or danger (Phelps and LeDoux, 2005), has been described as crucial in emotional reactivity (Pine, 2007). The amygdala has been deemed the critical brain region of this limbic system, for driving these processes (Robinson *et al.*, 2010). In their successful effort to find a well-fitting model of limbic circuitry, Stein *et al.* (2007) found the amygdala to be interconnected with the parahippocampal gyrus, subgenual cingulate, orbitofrontal cortex, posterior cingulate, and supragenual cingulate (Stein *et al.*, 2007). In another study, areas showing connectivity with the amygdala included medial prefrontal regions, insula, thalamus, striatum, and dorsal and posterior regions (Roy *et al.*, 2009). The dorsal ACC (dACC), along with the anterior insular cortices, constitutes an important element of the salience network, which serves to evaluate the relevance of internal and external stimuli in order to generate appropriate responses and guide behaviour (Seeley *et al.*, 2007). This network has not been studied in adolescent depression and anxiety before. This is remarkable, given the relevance of regions of the ACC (such as the pregenual and rostral ACC) to depression and anxiety in adults (Freitas-Ferrari *et al.*, 2010, Etkin *et al.*, 2010) as well as in youth (the subgenual ACC) (McClure *et al.*, 2007, Cullen *et al.*, 2009, Gaffrey *et al.*, 2010, Jiao *et al.*, 2011, Jin *et al.*, 2011, Connolly *et al.*, 2013). RSFC abnormalities of the default mode network (DMN) have been found in various neuropsychiatric disorders, including depression and anxiety, in adults (Broyd *et al.*, 2009, Sylvester *et al.*, 2012). A recent RSFC study in children with preschool onset depression also

found aberrancies in DMN connectivity, reporting decreased connectivity between the posterior cingulate cortex (PCC) and temporal and parietal cortical areas as well as the cerebellum, and increased connectivity between the PCC and subgenual anterior cortical areas (Gaffrey *et al.*, 2012).

Studies investigating the developmental course of resting-state connectivity networks observed a change in connectivity patterns of these networks over time. More diffuse patterns were reported in childhood with greater connectivity between regions that were anatomically close to one another, and cohesive and more integrated networks were shown in adulthood with increased connectivity between areas that were spatially remote (Fair *et al.*, 2007, Fair *et al.*, 2008, Fair *et al.*, 2009, Kelly *et al.*, 2009, Supekar *et al.*, 2009, Jolles *et al.*, 2011). Adolescents showed an intermediate pattern of functional connectivity. Noting that the greatest developmental effects occur in networks associated with social and emotional functions, the importance of understanding maturational neural processes in young psychiatric populations was again highlighted (Kelly *et al.*, 2009).

In the present study we set out to investigate RSFC in a sample of treatment-naïve, clinically depressed adolescents and pair-wise matched controls. We opted for a seed-based correlation approach. As opposed to previous RSFC studies, we aimed to investigate RSFC not just in one, but in several networks putatively involved in depression and anxiety in concert: the limbic network, the DMN, and the salience network. Based on reports of functional connections between the amygdala and prefrontal areas (Roy *et al.*, 2009) and altered activity patterns in adolescent depression and anxiety (Pine, 2007, Hulvershorn *et al.*, 2011, Roy *et al.*, 2013), we hypothesised decreased RSFC for the limbic network, in particular between the amygdala and the prefrontal cortex, especially the ACC. Furthermore, previous task fMRI research has indicated an attentional bias to negative stimuli in depressed individuals (Maalouf *et al.*, 2012), which in turn has been linked to involvement of the ACC (Anand *et al.*, 2005, van Tol *et al.*, 2012). Decreased coupling between the dACC and the orbitofrontal cortex (OFC) has been reported in depressed adults, which was proposed to have an association with a negative processing bias in depression (Frodl *et al.*, 2010). We therefore hypothesised abnormalities of RSFC for the salience network, particularly decreased connectivity with the OFC. Based on previous literature, we also hypothesised abnormalities in DMN RSFC

in our sample of clinically depressed adolescents, especially increased connectivity with the subgenual ACC.
In addition, we expected greater aberrancies in RSFC to be associated with greater severity of depressive and anxiety symptoms.

Method

Participants

Fifty-two adolescents (26 clinically depressed, 26 controls) were selected as part of the EPISCA study (Emotional Pathways' Imaging Study in Clinical Adolescents). EPISCA is a longitudinal MRI study in which adolescents with clinical depression and healthy controls were followed over a six-month period (January 2010 till August 2012) (also see (Aghajani *et al.*, 2013)). The current study reports on cross-sectional data from both groups that were collected prior to treatment.

Inclusion criteria for the depressed group were: having clinical depression as assessed by categorical and dimensional measures of DSM-IV depressive and anxiety disorders (see below), no current and prior use of antidepressants, and being referred for CBT at an outpatient care unit of two child and adolescent psychiatric institutes. The clinically depressed adolescents were included within two weeks after initial screening. None of the participants had started any form of treatment prior to inclusion, and therefore none used medication. Inclusion criteria for the control group were: no current or past DSM-IV classifications, no clinical scores on validated mood and behavioural questionnaires, no history of traumatic experiences, and no current psychotherapeutic and/or psychopharmacological intervention of any kind. Exclusion criteria for all participants were: primary DSM-IV clinical diagnosis of ADHD, pervasive developmental disorders, post-traumatic stress disorder, Tourette's syndrome, obsessive-compulsive disorder, bipolar disorder, and psychotic disorders; current use of psychotropic medication; current substance abuse; history of neurological disorders or severe head injury; age <12 or >21 years; pregnancy; left-handedness; a full-scale intelligence score (FIQ) <80; and general MRI contraindications. Clinically depressed adolescents and controls were pair-wise matched by age, gender and FIQ.
From the original total group of 59 adolescents (29 clinically depressed, 30 controls), four participants (two depressed, two controls) were excluded after preprocessing (see below) from further analysis due to movement

>3 mm, two (one depressed, one control) because of anomalies found on their anatomical scan after inspection of all subjects' structural images by a neuroradiologist, and one control was excluded because he could not be pair-wise matched. Consequently, 52 participants were included in this study: 26 clinically depressed, treatment-naïve adolescents (mean age 15.4 ± 1.5) and 26 healthy controls (mean age 14.7 ± 1.5). Participants were scanned within two weeks of initial screening, and all were new to MRI scanning procedures.
The study was approved by the Medical Ethics Committees of the Leiden University Medical Centre and written informed consent was obtained from the participants and their parents.

Clinical measures

For all participants, several clinical measures were used for dimensional and categorical assessment of DSM-IV disorders. After the clinical assessment by child and adolescent psychiatrists, categorical DSM-IV diagnoses were further assessed with the child and parent versions of the Anxiety Disorders Interview Schedule (ADIS) (Silverman and Albano, 1996). The ADIS is a semi-structured interview to obtain DSM-IV-based classifications of anxiety and related disorders in children and adolescents, which has excellent reliability for combined diagnoses with kappa values spanning from .82 - .90 (Silverman *et al.*, 2001), as well as excellent interrater agreement for principal diagnosis ($\kappa = .92$) and individual anxiety disorders ($\kappa = .80 – 1.0$) (Lyneham *et al.*, 2007). Additionally, the Children's Depression Inventory (CDI) (Kovacs, 1992b), the Revised Child Anxiety and Depression Scale (RCADS) (Chorpita *et al.*, 2000), the Youth Self Report (YSR) (Achenbach, 1991d), and its parent version the Child Behaviour Check List (CBCL) (Achenbach, 1991b) were used to dimensionally assess the severity of symptoms. For a detailed description of these instruments, see Appendix 1. All participants were tested with Dutch versions of the Wechsler Intelligence scales for children (Wechsler, 1991b) or adults (Wechsler, 1997b). For the controls, the same instruments were used. Controls were excluded when they fulfilled criteria for a DSM-IV disorder on the ADIS, or when they had clinical or high subclinical scores on the questionnaires.

Image data acquisition

Image acquisition took place at the Leiden University Medical Centre.

Images were obtained on Philips 3T magnetic resonance imaging systems (Philips Healthcare, Best, The Netherlands), equipped with a SENSE-8 head coil. Prior to scanning, all participants were introduced to the scanning situation by lying in a dummy scanner and hearing scanner sounds. Resting-state functional MRI data were acquired at the beginning of a fixed imaging protocol for each subject, using T_2^*-weighted gradient-echo echo-planar imaging with the following scan parameters: 160 whole-brain volumes; repetition time 2200 ms; echo time 30 ms; flip angle 80°; 38 transverse slices; no slice gap; field of view 220 mm; in-plane voxel size 2.75 × 2.75 mm; slice thickness 2.72 mm; total duration of the resting-state run 6 minutes. In the darkened MR room participants were instructed to lie still with their eyes closed and not to fall asleep. After completion of the scan, subjects confirmed wakefulness during acquisition. A sagittal 3-dimensional gradient-echo T_1-weighted image was acquired for registration purposes and grey matter analysis with the following scan parameters: repetition time 9.8 ms; echo time 4.6 ms; flip angle 80°; 140 sagittal slices; no slice gap; field of view 224 mm; 1 mm isotropic voxels; duration 4:56 minutes.

Preprocessing

FMRI data preprocessing and statistics were carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Smith *et al.*, 2004) and were similar to previous studies by our group (Pannekoek *et al.*, 2013c, van der Werff *et al.*, 2013). The following pre-statistics processing was applied: motion correction using MCFLIRT, resulting in an estimation of the rigid-body motion at each time point relative to the reference volume chosen from the middle time point of the series; non-brain removal; spatial smoothing using a 6 mm full-width at half-maximum Gaussian kernel; grand mean scaling of the entire 4D dataset by a single multiplicative factor; high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with a 0.01 Hz cut-off). Registration of the resting-state data to high resolution T_1-weighted, and the T_1 to the 2mm isotropic MNI-152 standard space image (T_1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montréal, QC, Canada) was carried out. The resulting transformation matrices were then combined to obtain a native to MNI space transformation matrix and its inverse (MNI to native space).

Statistical analysis

Demographic and clinical characteristics were analysed using SPSS 20.0 (SPSS Inc., Chicago, Illinois) using an independent-samples t-test with significance set at $p<0.05$. Head movement in the scanner can have an impact on the time courses of RSFC (Satterthwaite *et al.*, 2012, Power *et al.*, 2012, Satterthwaite *et al.*, 2013), especially in youth and patient samples (Van Dijk *et al.*, 2012). Motion in the scanner was therefore compared between groups by analysing the mean absolute and relative displacement in mm in SPSS, which were obtained for each participant during preprocessing through MCFLIRT (Jenkinson *et al.*, 2002).

Based on previous literature, the following seed regions-of-interest were selected: bilateral amygdala (for the limbic network), bilateral dACC (for the salience network), and bilateral posterior cingulate cortex (PCC) (for the DMN). A mask was created in standard space for the amygdala based on the Harvard-Oxford Subcortical Structural Probability Atlas in FSL (Veer *et al.*, 2011). A study on mapping the functional connectivity of the ACC showed that the connectivity pattern of the salience network can be generated using a seed in the dACC (Margulies *et al.*, 2007). The coordinates for the dACC seeds were obtained from table 1 of this study. The voxel most reliably located in a given region make the greatest contribution to its signal (Roy *et al.*, 2009). In the pioneering study investigating DMN connectivity in major depression, Greicius *et al* (2003) demonstrated that a seed region-of-interest in the PCC generated the DMN (Greicius *et al.*, 2003). We therefore chose the peak voxel of the PCC in the DMN in their study as the seed for our DMN analysis. The coordinates for all seeds are displayed in Table 2. Spheres of 4 mm radius were created around the seed voxels. They were then transformed to native space by applying the inverse transformation matrix obtained from the registration procedure, and spatially averaged time series were extracted for each seed and for each subject. For each participant, and for each network of interest, we performed a multiple regression analysis using the general linear model (GLM) (as implemented in FEAT (Smith *et al.*, 2004)). The time courses that were extracted from the voxels in our seed regions were entered as a regressor in a GLM for each network. Apart from two regressors describing the left and right seeds, nine nuisance regressors were included in the model: signal from the white matter, cerebrospinal fluid signal and the global signal, as well as six motion parameters (three translations and three rotations). The global signal was included to reduce

the influence of artefacts caused by physiological signal sources (i.e. cardiac and respiratory) (Fox and Raichle, 2007).

Table 2. MNI Coordinates of the Seed Regions

Seed region	MNI coordinates		
	x	*y*	*z*
Amygdala	+/- 22	-6	-16
dACC	+/- 6	18	28
PCC/precuneus	+/- 2	-52	26

MNI = Montreal Neurological Institute; dACC = dorsal anterior cingulate cortex; PCC = posterior cingulate cortex

After reslicing the resulting parameter estimate maps and their corresponding within-subject variance maps into 2 mm isotropic MNI space, they were entered into a higher level within and between groups mixed effects analysis (one- and two-sample t-test). Adolescence is a period of continued neural development, making this a highly sensitive time that is characterized by major as well as minor developmental changes in the brain, which have been demonstrated in grey matter, white matter, and functional activation (Blakemore, 2012). Therefore, age was entered as a regressor. For each subject grey matter density maps were derived from the anatomical scans using FSL. As studies have indicated structural abnormalities in childhood anxiety and depression (Pine, 2007, Hulvershorn *et al.*, 2011), we used grey matter density information of each subject as a voxel-dependent covariate in our higher level model. Additionally, a previous study by our group in this sample indicated grey matter differences between the clinically depressed adolescents and healthy controls (Pannekoek *et al.*, 2014b). By including structural information in the functional connectivity analysis, variance explained by potential differences in grey matter density and/or possible misregistrations are taken into account (Oakes *et al.*, 2007). To correct for multiple comparisons, cluster correction was applied in all group analyses with significance set at a corrected $p<.05$ and an initial cluster-forming threshold of $Z>2.3$ (Worsley, 2001).

Table 1. Demographic and Clinical Characteristics of the Sample

Characteristic	Depressed adolescents *N* = 26	Controls *N* = 26
Age (Mean ± SD)	15.4 ± 1.5	14.7 ± 1.5
Sex (*N* male/*N* female)	3/23	3/23
IQ (Mean ± SD)	104.2 ± 8.7	106.6 ± 7.8
CDI [a] (Mean ± SD)	18.6 ± 9.5**	4.6 ± 3.4**
RCADS—Depression[b] (Mean ± SD)	11.2 ± 5.7**	3.9 ± 3.0**
RCADS—Anxiety[b] (Mean ± SD)	32.7 ± 14.6**	14.8 ± 10.8**
YSR—Internalising (Mean ± SD)	24.0 ± 8.7*	8.3 ± 6.3*
YSR—Externalising (Mean ± SD)	12.5 ± 6.7**	6.7 ± 5.8**
CBCL—Internalising (Mean ± SD)	19.3 ± 7.5**	3.9 ± 3.6**
CBCL—Externalising[b] (Mean ± SD)	10.7 ± 9.3*	3.5 ± 4.0*
- Anxiety disorder	23	0

Because less than 20% of the items in CDI, RCADS, YSR and CBCL were missing, expectation maximizatio regression method was used to calculate the scale scores. IQ = Intelligence quotient; CDI = Children's Depres Inventory; RCADS = Revised Child Anxiety and Depression Scale; YSR = Youth Self Report; CBCL = Child Behav Check List.

[a] One patient did not complete the questionnaire

[b] Three clinically depressed adolescents and their parents/primary caregivers did not complete the question

* $p<0.05$; ** $p<0.001$

Results

Demographics and clinical characteristics

The 26 clinically depressed adolescents all had clinical depression scores on the categorical and/or dimensional measurements, with 18 having a comorbid anxiety disorder. All were treatment-naïve for pharmacotherapy and psychotherapy. The groups did not differ on age (U= 438.0; p= .062) and FIQ (t= 1.026; p= .310). Clinically depressed adolescents showed significantly higher scores on CDI, CBCL, and RCADS depression and total anxiety scales (p<.001), and on YSR scores (p< .05) (Table 1). The groups did not differ on mean absolute displacement (p= .392) and mean relative displacement (p=.570) as measures for head motion in the scanner. Information on motion is displayed in Supplementary Table S1.

RSFC analysis

Analysis of amygdala RSFC showed that the connectivity pattern within both groups largely comprised areas described to have functional and anatomical connections with the amygdalae in previous work (Stein *et al*., 2007, Roy *et al*., 2009). Areas showing positive connectivity with the amygdala seeds included the hippocampus, parahippocampal gyrus, middle temporal gyrus, thalamus, putamen, insular cortex, frontal pole and the brain stem. Areas showing negative RSFC included the cuneus, precuneus, PCC, angular gyrus, supramarginal gyrus, and lateral occipital cortex.

With respect to between-group analysis for the limbic network, we found increased positive left amygdala RSFC in the depressed group with the right middle frontal gyrus, inferior frontal gyrus, precentral gyrus (bordering white matter), and the postcentral gyrus relative to controls (Figure 1A; Table S2). We also found decreased negative right amygdala RSFC in depressed adolescents with the left frontal pole, right pregenual ACC, right paracingulate gyrus, and the right superior frontal gyrus, as well as with the left angular gyrus, left lateral occipital cortex, and the left supramarginal gyrus (Figure 1B; Table S2).

No group differences were found for the contrast with both left and right amygdala combined.

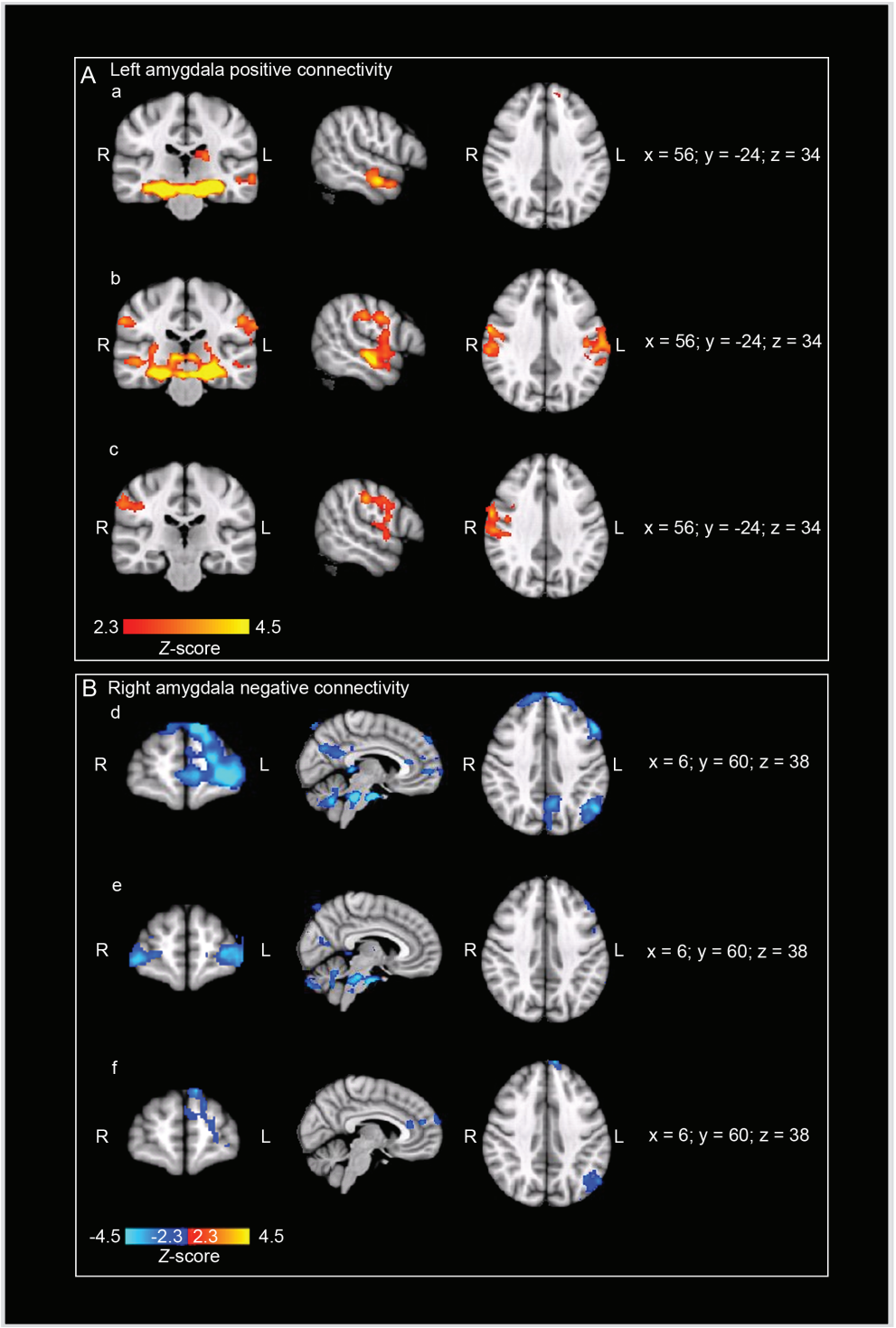

A Left amygdala positive connectivity
a
R
L
R
L
x = 56; y = -24; z = 34
b
R
L
R
L
x = 56; y = -24; z = 34
c
R
L
R
L
x = 56; y = -24; z = 34
2.3
4.5
Z-score
B Right amygdala negative connectivity
d
R
L
R
L
x = 6; y = 60; z = 38
e
R
L
R
L
x = 6; y = 60; z = 38
f
R
L
R
L
x = 6; y = 60; z = 38
-4.5
-2.3
2.3
4.5
Z-score

Figure 1.
Left (A) and right (B) amygdala connectivity. A (a) main effect for the control group in left amygdala positive connectivity; (b) main effect for the clinically depressed group in left amygdala positive connectivity; (c) the difference in left amygdala positive connectivity between the two groups. B (d) main effect for the control group in right amygdala negative connectivity; (e) main effect for the clinically depressed group in right amygdala negative connectivity; (f) the difference in right amygdala negative connectivity between the two groups. Images are z-statistics, overlaid on the MNI-152 standard brain. Cold colors indicate negative connectivity, warm colors indicate positive connectivity. The left hemisphere of the brain corresponds with the right side of the images. MNI coordinates displayed at the right side of the images correspond with the coordinates of the displayed slices.

The dACC seeds probing the salience network showed similar connectivity patterns in both groups, corresponding with previous literature (Margulies *et al.*, 2007). Areas showing positive RSFC included other parts of the ACC, the paracingulate gyrus, frontal pole, insular cortex, temporal pole, precentral gyrus, supramarginal gyrus, middle frontal gyrus, and inferior frontal gyrus. Areas showing negative connectivity at rest included the PCC, precuneus, angular gyrus, lateral occipital cortex, middle frontal gyrus, superior frontal gyrus, inferior temporal gyrus, and the frontal pole. In the between-group analyses, the combined bilateral dACC in a single contrast showed decreased positive connectivity in clinically depressed adolescents with the right middle frontal gyrus, frontal pole, and inferior frontal gyrus (Figure 2; Table S3). Separate investigations of the left and right dACC did not produce significant group differences.

We investigated DMN activity with seeds in the left and right PCC/precuneus. Similar connectivity patterns in both groups were observed with the following areas showing positive RSFC: the PCC/precuneus, cuneus, superior lateral occipital cortex, angular gyrus, supramarginal gyrus, frontal pole, superior frontal gyrus, paracingulate gyrus, ventral ACC, and the subcallosal cortex. Negative RSFC patterns included the dACC, supplementary motor cortex, precentral gyrus, postcentral gyrus, central opercular cortex, insular cortex, inferior lateral occipital cortex, and the occipital pole (Figure S1). No group difference was found in DMN

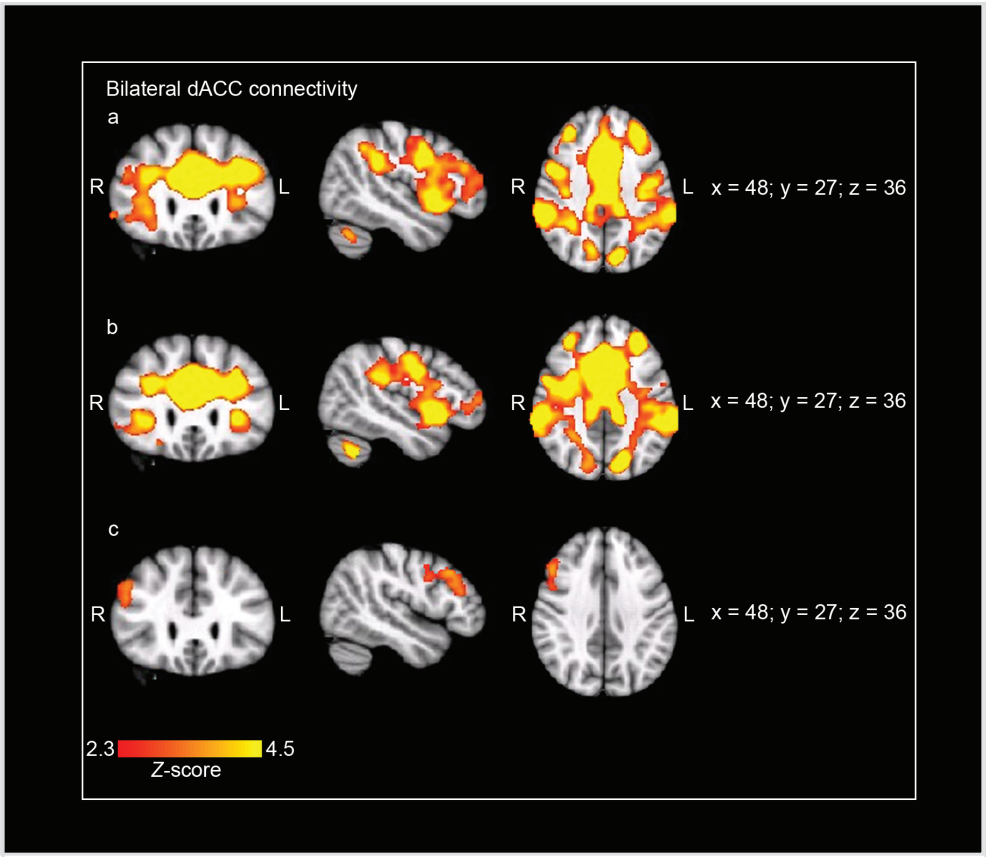


Figure 2.
Bilateral dorsal anterior cingulate cortex connectivity. (A) main effect for the control group in bilateral dorsal anterior cingulate positive connectivity; (B) main effect for the clinically depressed group in bilateral dorsal anterior cingulate positive connectivity; (C) the difference in bilateral dorsal anterior cingulate positive connectivity between the two groups. Images are z-statistics, overlaid on the MNI-152 standard brain. The left hemisphere of the brain corresponds with the right side of the images. Yellow to red are z-values, ranging from 2.3 to 4.5. MNI coordinates displayed at the right side of the images correspond with the coordinates of the displayed slices.

connectivity.

Additional analyses were carried out to determine whether the observed differences in RSFC were unchanged after removing the grey matter covariate. Analyses without correcting for grey matter differences yielded the same results as the analyses including the voxel-dependent covariate.

Symptom severity and RSFC

To test our hypothesis about an association between severity of depressive and anxiety symptomatology and RSFC abnormalities in the depressed group, RSFC data were correlated with depression and total anxiety scores on the RCADS using SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA). Data points three or more standard deviations from the mean were considered outliers. One outlier was excluded from the analyses with total anxiety scores. We generated the mean z-values of the clusters resulting from each of our seed-based resting-state analyses; i.e. the individual connection between the seed and the regions of difference. For example, we created a mask of the areas resulting from our bilateral dACC analysis, and calculated the individual mean Z-scores from these areas using Featquery, part of FSL (Smith *et al.*, 2004). Hierarchical multiple regressions were used, corrected for age, IQ and gender across all subjects. To correct for multiple comparisons in the regression analyses, a Bonferroni-corrected p-value was generated based on the two RCADS scales used (depression and the total of all anxiety scales) as well as the number of regions in this analysis. We followed the same approach for the areas showing aberrant connectivity in our left and right amygdala analyses. We found a positive correlation between connectivity strength and depression scores on the RCADS in the areas that showed decreased RSFC with the bilateral dACC in depressive participants compared to typically developing adolescents (unstandardized b = 3.006, $t(21)$ = 3.253, p = .004). No significant associations were found between connectivity of any of the other regions showing abnormal RSFC and depression and anxiety severity scores in the clinically depressed group (Table S4). Similar analyses within the controls did not yield any associations between RSFC and severity scores.

Discussion

In this study of clinically depressed treatment-naïve adolescents and pair-wise matched controls, RSFC was examined in limbic, salience and default mode networks. We also investigated whether RSFC deviations were associated with depression and anxiety scores in the depressed group. As hypothesised, clinically depressed adolescents showed RSFC differences in the limbic and salience networks, but contrary to expectation not in the DMN. Greater abnormalities in RSFC were not associated with higher clinical ratings of depression and anxiety.

Our findings are partly in line with other RSFC studies investigating adolescent depression, showing abnormalities in connectivity in networks involving the amygdala, ACC, inferior frontal areas, and prefrontal areas (Jiao *et al.*, 2011, Jin *et al.*, 2011, Luking *et al.*, 2011). These brain regions were also implicated in task studies investigating depression and social anxiety in children and adolescents (Guyer *et al.*, 2008, Halari *et al.*, 2009). A novel finding for the limbic network in clinically depressed adolescents is the increased left amygdala RSFC with right cortical areas, including the middle frontal gyrus, inferior frontal gyrus, precentral gyrus, and postcentral gyrus. It is known that these areas are involved in emotion (Grecucci *et al.*, 2013), and we offer a cautious interpretation. Interestingly, building on prior findings that suggested left and right amygdala involvement in different functions (Phelps *et al.*, 2001, Goldin *et al.*, 2008), a study by Dyck *et al.* (2011) found evidence for lateralization of functions of the amygdala using visual and audiovisual mood induction paradigms, supporting a left-lateralized cognitive and intentional control of mood. The findings of their study implicated that responses of the left amygdala are modulated by subjective experience, and may reflect variations in the regulation of mood (Dyck *et al.*, 2011). Relationships between cognitive emotion regulation strategies and depressive symptoms in adolescent samples have been previously reported (Garnefski and Kraaij, 2006). In our study of clinically depressed adolescents, an increased RSFC between the left amygdala and the right cortical areas could reflect or underlie an altered involvement of the left amygdala in conscious and cognitively controlled emotion processing in adolescent depression. However, this interpretation remains speculative, as no measures of emotion regulation were used in the present study.

In agreement with prior studies and our hypothesis, we also found decreased RSFC of the right amygdala with prefrontal areas, notably the pregenual ACC (Etkin *et al.*, 2010, Carballedo *et al.*, 2011). This is in line with previous literature linking connectivity between the amygdala and the pregenual ACC to the regulation of affective processing (Etkin *et al.*, 2011b). Furthermore, previous studies have indicated that the ACC is involved in emotion regulation by having an inhibitory effect on amygdala function, which is consistent with the hypothesis that decreased cortical regulation of limbic activation may be present in depression (Phelps and LeDoux, 2005, Anand *et al.*, 2005). Taken together this suggests that the

decrease of RSFC between the right amygdala and the ACC could reflect or underlie diminished emotion regulation.

In the salience network we found a decreased positive connectivity between the bilateral dACC and right prefrontal regions in clinically depressed adolescents. We did not find an altered coupling between the dACC and the OFC, as was hypothesised. However, altered RSFC of the ACC with frontal cortical regions has been reported in adult depression (Sheline *et al.*, 2010). Several functions attributed to the right dorsolateral prefrontal cortex (dlPFC) and the ACC and their connections may be relevant for the interpretation of our results. For instance, within the scope of the salience network, one of the roles of the dACC is mediating the integration of information across events. An attention bias to negative emotions has been reported in clinical depression (Gotlib *et al.*, 2004), and research findings indicate that this bias is state-dependent, as it was found to be present in depressed adolescents but absent in remission (Maalouf *et al.*, 2012). This suggests that abnormal connectivity between these areas may be related to the increased negative self-attribution and biases in information processing typically observed in MDD patients (Grimm *et al.*, 2009). This hypothesis is further strengthened by the association we found between the depression scores on the RCADS and dACC connectivity. Adolescent depression has previously been associated with a bias towards negative emotional stimuli (Maalouf *et al.*, 2012). With respect to the role of the salience network of identifying personally relevant stimuli among the myriad inputs (Seeley *et al.*, 2007), this bias is plausible in depressed adolescents. Aberrant functioning of the salience network could play a role in a heightened sensitivity to negative stimuli in depressed adolescents. Finally, prior work has shown that dACC connectivity is related to affective processing (Adelstein *et al.*, 2011), and that the PFC also plays a role in affective working memory and the representation of positive and negative affect states (Grimm *et al.*, 2009). Thus, aberrant coupling between the ACC and dlPFC could also represent or underlie a defective evaluation of negative emotion. Taken together, for the salience network, abnormal connectivity between the dACC and prefrontal regions may be specifically related to disturbed assessment of affective stimuli.

In contrast to prior findings in adult depression, (Greicius *et al.*, 2007, Broyd

et al., 2009, Wang, 2012), we did not find differences in DMN connectivity. A study comparing RSFC between healthy children and young adults found that the size of functional connectivity (number of voxels) and RSFC strength differed between the two groups, independent of grey matter differences (Jolles *et al*., 2011). This also specifically applied to the DMN (Fair *et al*., 2008, Supekar *et al*., 2010). These results indicate an ongoing change of functional connectivity during adolescence. It could be argued that the inconsistency between findings of DMN RSFC abnormalities in adult depression and the lack of DMN RSFC abnormalities in the present study may be linked to such developmental differences. However, DMN connectivity abnormalities have also been reported by studies in depressed non-adult samples, for example by Gaffrey *et al*., who reported DMN connectivity in children with a history of preschool depression prior to the age of 6 (Gaffrey *et al*., 2012). Another study of depressed adolescents recently found altered RSFC of the subgenual ACC, a region linked to the DMN, and an associated increased rumination and higher depression levels in depressed subjects (Connolly *et al*., 2013). These discrepancies with our findings might be due to differences in study samples and methodology, like age ranges and inclusion of various comorbid conditions.

We believe our study has several strengths. We studied a substantial sample of clinically depressed, treatment-naïve adolescents, and investigated three networks putatively involved in affective disorders with a seed-based approach, allowing easier replicability (Cole *et al*., 2010). However, several limitations should also be noted. By using seed-based correlation instead of a data-driven approach, possible RSFC abnormalities in other resting-state networks might be overlooked. Also, the interpretation of abnormalities in RSFC in a cross-sectional observational design cannot be conclusive in terms of causality and should be done with caution. It is also of note that we only explored the functional connectivity of specific key regions of each network. Future studies should explore other seed regions or networks. Finally, anxiety co-occurred with depression in most of our clinically depressed adolescents, which could affect the specificity of the results. However, other studies investigating adolescent depression reported a similar comorbidity, and comorbidity with anxiety is considered to be characteristic for clinical depression in adolescence. Exclusion of anxiety comorbidity would therefore have resulted in an atypical sample (Zahn-Waxler *et al*., 2000, Cullen *et al*., 2009).

Conclusion

In summary, as expected, abnormalities were found in RSFC of the limbic and salience networks in clinically depressed adolescents. However, our results did not support the hypothesis that DMN RSFC is deviant. The aberrant RSFC of the amygdala network and dACC network may be related to altered emotion regulation and self-referential processing in depressed adolescents. Taken together, our results could provide new insights into disturbances of functional connectivity in depressed adolescents and suggest that models for affective disorders in adolescents may include abnormalities in the connectivity of the salience network. Clearly, future studies are needed to replicate the current findings and to further explore RSFC in adolescent depression.

Appendix and supplementary information

Appendix S1. Description of clinical measures

For all participants, several clinical measures were used for categorical and dimensional assessment of DSM-IV disorders. After clinical assessment of the patients by child and adolescent psychiatrists, categorical DSM-IV diagnoses were further assessed with the child and parent versions of the Anxiety Disorders Interview Schedule (ADIS) (Silverman and Albano, 1996). Additionally, the Children's Depression Inventory (CDI) (Kovacs, 1992b), the Revised Child Anxiety and Depression Scale (RCADS) (Chorpita *et al.*, 2000), the Youth Self Report (YSR) (Achenbach, 1991d) and its parent version the Child Behaviour Check List (CBCL) (Achenbach, 1991b) These instruments were also used to assess the healthy controls.

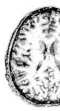
The ADIS is a structured diagnostic interview consistent with the DSM-IV criteria for depressive and anxiety disorders. The child and parents are interviewed separately and based on the outcomes of these interviews a final clinical severity score is given by the interviewer. The ADIS is shown to have good psychometric properties and was used in this study by trained clinicians and researchers.

The CDI is a self-report questionnaire with 27 items that correspond with DSM-IV dimensions of depressive disorders, and is scored on a 3-point Likert scale describing the severity of symptoms (0 = *absence* of symptomatology to 2 = *severe symptomatology*).

The RCADS is a self-report questionnaire with 47 items that

correspond with DSM-IV dimensions of depressive and anxiety disorders. The items are descriptive statements that are scored on a 4-point Likert scale (0 = *never* to 3 = *always*). The questionnaire covers six scales, corresponding to DSM-IV dimensions of anxiety and depressive disorders: Separation Anxiety Disorder (SAD), Generalized Anxiety Disorder (GAD), Social Phobia (SP), Major Depressive Disorder (MDD), Panic Disorder (PD), and Obsessive Compulsive Disorder (OCD). In the present study, only two scale scores were used: the total anxiety score (sum of the five scale scores about the anxiety disorders SAD, GAD, SP, PD and OCD) and a depression score (the MDD scale score). Because only three items were missing, expectation maximization as regression method was used to calculate the scale scores. The internal consistencies of these scale scores in both groups available (three were missing in the depressed group) are high (.93/.93 for the total anxiety score and .74/.86 for the depression score in respectively the patient and control groups).

The YSR covers 113 items concerning behavioural and emotional problems in the past 6 months, as reported by the adolescent. The internalising scale of the YSR contains 31 items in the form of descriptive statements that are scored on a 3-point Likert scale (0 = *not true* to 2 = *very or often true).*
The CBCL covers 118 items concerning behavioural and emotional problems in the past 6 months, as reported by parents or primary caregivers. The internalising scale of the CBCL contains 33 items in the form of descriptive statements that are scored on a 3-point Likert scale (0 = *not true* to 2 = *very or often true*).
All participants were tested with Dutch versions of the Wechsler Intelligence scales for children (Wechsler, 1991b) or adults (Wechsler, 1997b).



Supplementary tables S1 – S4

Supplementary Table 1. Group comparison for motion parameters

Statistic	Depressed adolescents	Controls
N	26	26
Mean (SD) AD	0.2100 (0.09011)	0.0657 (0.01550)
Minimum AD	0.07	0.06
Maximum AD	0.48	0.44
Mean (SD) RD	0.0735 (0.02560)	0.0657 (0.01550)
Minimum RD	0.04	0.05
Maximum RD	0.13	0.11
	Absolute displacement	Relative displacement
Mann-Whitney U	441.000	307.500
p	.059	.570

AD = absolute displacement in mm; RD = relative displacement in mm

Supplementary Table 2. Amygdala Resting-State Connectivity, group differences

Left amygdala RSFC[1]: DEP[2] > HC[3]

Cluster 1, 1785 voxels	Side	*Z*-value	*p*-value	MNI[4] Coordinates		
Region				*x*	*y*	*z*
Middle Frontal Gyrus	R	4.0816	0.0001	36	16	28
Precentral Gyrus	R	4.04997	0.0001	60	-4	34
Postcentral Gyrus	R	3.94122	0.0001	56	-20	38
Precentral Gyrus	R	3.86925	0.0001	38	0	30
Supramarginal Gyrus, BA[5] 3	R	3.71335	0.0002	62	-20	40
White Matter	R	3.55519	0.0004	42	-8	24

Right amygdala RSFC: HC[3] > DEP[2]

Cluster 2, 977 voxels	Side	*Z*-value	*p*-value	MNI[4] Coordinates		
Region				*x*	*y*	*z*
Frontal Pole	L	4.10506	0.0001	-8	60	38
Frontal Pole	L	4.09019	0.0001	-14	64	26
Anterior Cingulate Gyrus, BA[5] 32	R	3.81057	0.0001	14	40	12
Frontal Pole	L	3.53309	0.0004	-8	68	24
Paracingulate Gyrus, BA[5] 9	R	3.51748	0.0004	6	48	18
Frontal Pole, BA[5] 10	L	3.51675	0.0004	-20	58	14
Cluster 1, 639 voxels	Side	*Z*-value	*p*-value	MNI[4] Coordinates		
Angular Gyrus, BA[5] 39	L	3.65803	0.0003	-40	-58	32
Lateral Occipital Cortex, BA[5] 39	L	3.51779	0.0004	-52	-64	38
Lateral Occipital Cortex	L	3.35477	0.0008	-40	-64	38
Angular Gyrus	L	3.26345	0.0011	-48	-54	32
Lateral Occipital Cortex	L	3.14998	0.0016	-42	-72	34
Angular Gyrus	L	2.92358	0.0035	-56	-60	32

[1] RSFC = resting-state functional connectivity; [2] DEP = clinically depressed adolescents; [3] HC = healthy controls; [4] MNI = Montreal Neurologic Institute; [5] BA = Brodmann Area. Group differences are significant in all regions after correction for multiple testing over 3 network analyses (0.05/3 = p<0.017).

Supplementary Table 3. Bilateral dACC Resting-State Connectivity: HC[1] > DEP[2], group difference

Cluster 1, 609 voxels	Side	*Z*-value	*p*-value	MNI[3] Coordinates		
Region				*x*	*y*	*z*
Middle Frontal Gyrus	R	3.75842	0.0002	48	24	38
Middle Frontal Gyrus	R	3.67548	0.0002	50	32	22
Middle Frontal Gyrus, BA[4] 9	R	3.55386	0.0004	48	28	28
Frontal Pole	R	3.41668	0.0006	56	40	12
Precentral Gyrus	R	3.0845	0.0020	48	6	34
Middle Frontal Gyrus	R	2.70543	0.0068	54	12	42

[1] HC = healthy controls; [2]DEP = clinically depressed adolescents; [3] MNI = Montreal Neurologic Institute;
[4] BA = Brodmann Area.
Group differences are significant in all regions after correction for multiple testing over 3 network analyses (0.05/3 = p<0.017).

Supplementary Table 4. Associations between Clinical Scores and RSFC

	Depression scores			Anxiety scores		
Brain region	Unstandardized *b*	*t*	*p*	Unstandardized *b*	*t*	*p*
l amyg – r MFG	-1.211	-.585	.566	-.2033	-.327	.747
r amyg – r ACC	-1.481	-.805	.431	-3.855	-.699	.493
r amyg – l LOC	-1.351	-.968	.346	-.667	-.156	.878
bil dACC – r MFG	3.006	3.252	.004*	8.157	2.819	.011

RSFC = resting-state functional connectivity; l = left; r = right; amyg = amygdala; MFG = middle frontal gyrus; ACC = anterior cingulate cortex; LOC = lateral occipital cortex; bil = bilateral; dACC = dorsal ACC.
* = significant at Bonferroni corrected p < .006

Supplementary figure S1

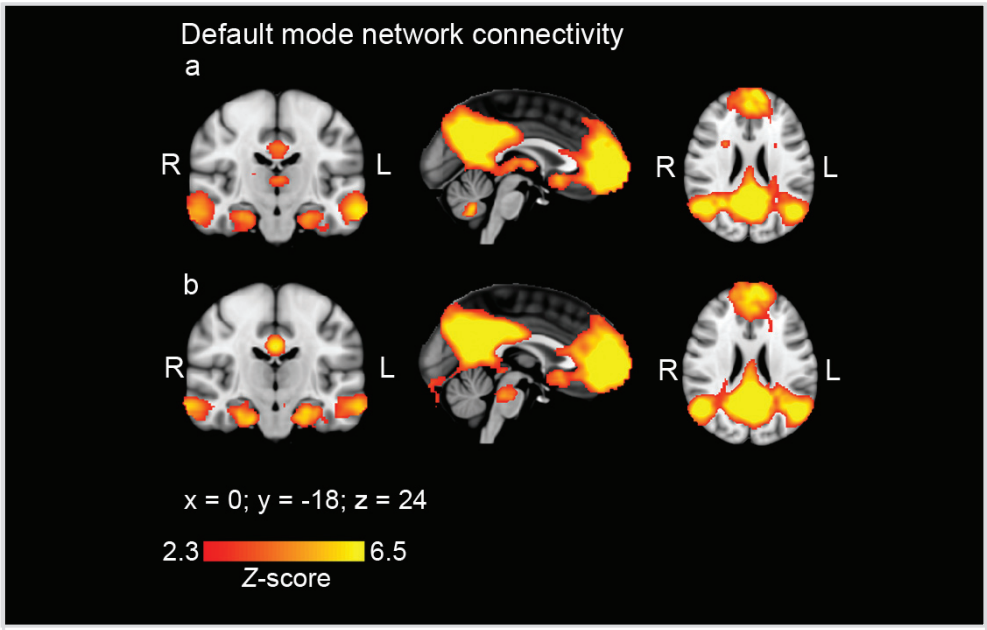


Figure S1. Default mode network connectivity
(a) main effect for the control group in default mode network connectivity; (b) main effect for the clinically depressed group in default mode network connectivity. Images are z¬-statistics, overlaid on the MNI-152 standard brain. The left hemisphere of the brain corresponds with the right side of the images. Yellow to red are z-values, ranging from 2.3 to 6.5. MNI coordinates displayed at the bottom of the figure correspond with the coordinates of the displayed slices.