The Affective Amygdala : towards a better understanding of adolescent depressive and anxiety disorders

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Chapter 6

Longitudinal changes in right amygdala – dorsomedial prefrontal cortex connectivity

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Longitudinal changes in resting-state functional connectivity in depressed and anxious adolescents in relation to treatment.

Abstract

Previous cross-sectional studies indicated differences in resting state functional connectivity (RSFC) between the amygdala and the prefrontal cortex (PFC) in adolescents with depressive and anxiety disorders. However, little is known about longitudinal changes in RSFC that occur during treatment. This study tested longitudinal changes in RSFC in 20 treatment-naïve adolescents (12-19 years old) with a depressive or anxiety disorder and 24 healthy control group adolescents who were group-wise matched on age and gender. All adolescents were scanned at two occasions, which were separated by a six-month period during which the adolescents from the clinical group received cognitive behavior therapy based treatment. We used a seed-based region-of-interest (ROI) approach with seeds in bilateral amygdala. There was a significant session x group interaction in which the clinical group showed an increase in positive connectivity between right amygdala and medial PFC over time. In addition, the change in connectivity was associated with change in self-reported depression symptoms in the complete sample: adolescents who showed a larger increase in positive connectivity also showed a larger decrease in depression symptoms. Results can be interpreted by an increase in top-down regulation by PFC regions, and suggests that receiving treatment for depressive or anxiety disorders is accompanied by changes in RSFC. Future research should further investigate treatment effects by including larger samples of adolescents with depressive and anxiety disorders that are referred for different forms of treatment, e.g. structured CBT procedures or medication.

Introduction

Depression and anxiety are two of the most common disorders diagnosed during adolescence (Costello et al., 2011; Merikangas et al., 2010). Approximately 6-15% of all adolescents get confronted with either a depressive or an anxiety disorder (Kessler et al., 2012a; Thapar, Collishaw, Pine, & Thapar, 2012). Depression and anxiety are both characterized by problems with affect regulation, which influences the adolescents' thoughts, feelings and behaviors (Zisook et al., 2007). The comorbidity between depression and anxiety is high (Essau, 2003; Essau, 2008; Simms, Prisciandaro, Krueger, & Goldberg, 2012) and having both disorders increases the risk for a negative outcome: people report more impairments (Lewinsohn et al., 1995), more severe internalizing problems (Beesdo, Knappe, & Pine, 2009a) and more severe emotional disturbances (Kessler et al., 2012b). Studying the neurobiological mechanisms of adolescent depression and anxiety might provide us with valuable information on de development and persistence of these disorders. Adolescence is a critical period in which brain development and the refinement of neuronal connections is still ongoing (Blakemore, 2012; Paus, 2005), which likely makes adolescents vulnerable for the onset of depressive and anxiety disorders (Casey et al., 2008; Gogtay et al., 2004; Somerville, & Casey, 2010). Studying the neurobiological mechanisms of adolescent depression and anxiety might thus provide us with valuable information on de development and persistence of these disorders.

Studies using task related functional MRI (fMRI) indicated that adolescents with depressive and anxiety showed increased amygdala reactivity to affective stimuli compared to healthy control adolescents (Mcclure et al., 2007b; Monk et al., 2008a; Roberson-Nay et al., 2006; Thomas et al., 2001a). It was also indicated that amygdala activation in response to affective stimuli increases after treatment (Maslowsky et al., 2010). Recently, interest rose in investigating functional connectivity in adolescents with depressive and anxiety disorders (Cullen et al., 2009; Gaffrey, Luby, Botteron, Repovs, & Barch, 2012; Jiao et al., 2011), by means of resting state functional connecti-

vity (RSFC; Biswal, Yetkin, Haughton, & Hyde, 1995; Fox, & Raichle, 2007). Results showed differences between depressed and anxious adolescents and healthy controls in RSFC between the amygdala and various sub regions of the medial prefrontal cortex. For example, a recent study from our group (Pannekoek et al., 2014a) found more positive RSFC between the limbic network with the amygdala as seed and i.e. the right middle frontal gyrus, and the inferior frontal gyrus. These connections were stronger for clinically depressed adolescents compared to healthy control adolescents (Pannekoek et al., 2014a). In addition, negative RSFC between the amygdala and medial PFC, including the anterior cingulate cortex (ACC) was found, which was less pronounced in the clinically depressed adolescents. These findings fit well with other studies that investigated RSFC in adolescents with depressive and anxiety disorders (Hulvershorn et al., 2011; Pine, 2007). It was also reported that there is a positive relation between the intensity of amygdala-centered connectivity regions and symptom severity, which might indicate that depression is related to dysregulation of functional connectivity in amygdale related brain networks (Jin et al., 2011). Finally, research by Roy and colleagues (Roy et al., 2013) examined RSFC in adolescents with generalized anxiety disorder (GAD). Their results indicated that adolescents with GAD showed disruptions in functional connectivity between amygdala and several PFC areas, including medial PFC, when compared to healthy adolescents. Adolescents with GAD showed negative connectivity for some regions, e.g. ventromedial PFC, and positive connectivity for other regions like the dorsomedial PFC (DMPFC). These results suggest that there is a distortion in functional connectivity between subcortical (e.g. amygdala) and cortical (e.g. medial PFC) regions that might cause depression and anxiety related symptomatology (Anand et al., 2005; Phelps, & Ledoux, 2005).

These cross-sectional studies provided us with important new insights on the neurobiological mechanisms of adolescent depression and anxiety and support the hypothesis of disturbed subcortical-cortical connectivity (Mayberg, 1997). However, very little is known about longitudinal

changes in RSFC in adolescents with depressive and anxiety disorders. It is important to investigate longitudinal RSFC in relation to depression/anxiety, changes in self-reported symptomatology and treatment outcome, to open avenues to increase treatment effectiveness and provide better guidelines for early intervention. Longitudinal studies provide us with the opportunity to detect individual changes, which is necessary to understand the influence of treatment on brain functioning and the development and persistence of psychiatric disorders (Crone, & Elzinga, 2014).

Therefore, the aim of the current study was to examine longitudinal changes in RSFC in adolescents with a DSM-IV depressive or anxiety disorder and healthy control group adolescents. For the adolescents with depression/anxiety disorders, data was acquired at two time points: before the start of their regular CBT-based treatment and six months after the start of the treatment. The control group adolescents were assessed in similar time periods. We used a seed-based approach with seeds in the bilateral amygdala. In line with previous research that reported disturbed amygdala-medial PFC connectivity in depression and anxiety (Anand et al., 2005; Phelps, & Ledoux, 2005) we expected to find a group x time interaction in RSFC between the amygdala and the prefrontal cortex. Furthermore, it was expected that adolescents who showed a larger change in symptoms as measured with self-report anxiety and depression questionnaires, would show a larger change in functional connectivity between amygdala and PFC.

Methods and Materials

Participants

The original study sample consisted of 61 participants at session 1 (Van Den Bulk et al., 2014), of which 17 were excluded for the current analyses due to various reasons (N=10 clinical; N=7 control): due to technical problems during scanning, contra indications for fMRI, poor data quality due to movement artifacts, anomalous findings reported by the radiologist, unfo-

reseen clinical features, or drop-out of the study because they were no longer interested or eligible (complex family problems, compulsory admission, broken contact).

The final sample consisted of 20 treatment-naïve adolescents with a clinical diagnosis of a DSM-IV depressive or anxiety disorder that were referred for CBT-based treatment (CLIN) and 24 healthy controls (CNTR) who completed 2 functional Magnetic Resonance Imaging (fMRI) sessions. FMRI data for the clinical group were collected before the start of regular CBT-based treatment (session 1) and six months (session 2) after session 1. The adolescents in the control group were scanned within the same time interval and did not receive treatment. Data used for this study is a selection from a larger study called EPISCA (Emotional Pathways' Imaging Study in Clinical Adolescents). EPISCA is a unique longitudinal studies investigating emotion processing in adolescents with depressive and/or anxiety disorders, adolescents who experienced childhood sexual abuse and healthy control group adolescents (Aghajani et al., 2013; Pannekoek et al., 2014a; Van Den Bulk et al., 2014).

Adolescents from the clinical group were recruited in outpatient departments of two child and adolescent psychiatric institutes. Adolescents in the control group were recruited through local advertisement, with the following inclusion criteria: no clinical scores on validated mood and behavioral questionnaires, no history of traumatic experiences, and no current psychotherapeutic intervention of any kind. All adolescents were between 12 and 19 years of age and had an estimated intelligence ≥80. Exclusion criteria for all participants were: any other primary DSM-IV diagnosis, current use of psychotropic medication (stable SSRI use was allowed; N=2), current substance abuse, a history of neurological disorders or severe head injury, left-handedness, and general MRI contraindications (e.g. metal implants, claustrophobia, and pregnancy).

There were no significant differences between the groups considering age and sex (Table 1). For all participants, estimated full-scale IQ scores were

acquired with six subtests of the Wechsler Intelligence Scale for Children-III or the Wechsler Adult Intelligence Scale (Wechsler, 1991; Wechsler, 1997). All participants scored within the average range and there was no significant difference between groups.

After complete description of the study to the participants, informed consent was obtained from all participants, and from a primary care giver for every participant under the age of 18. The adolescents received a financial compensation including travel expenses for their participation. The Medical Ethics Committee of the Leiden University Medical Center approved the study and all anatomical scans were reviewed and cleared by a radiologist.

Table 1. Participant characteristics of adolescents with a depressive/anxiety disorder and healthy control group adolescents

	Clinical		**Control**				
	N		**N**		χ^2	**df**	***p***
N	20		24				
Females/Males	19/1		20/4		1.47	1	.36
	Mean	**SD**	**Mean**	**SD**	**t**	**df**	***p***
Age session 1	15.81	1.48	15.35	1.65	-.96	42	.34
Full scale IQ	106	8.00	107	8.01	.50	42	.62
Weeks between sessions							
Session 1 – Session 2	29.00	2.90	28.83	2.58	-.20	42	.84
Session 1							
DSM-IV Classification:	**N**	**%**	**N**	**%**			
No disorders	0	0	24	100			
Depression	6	30					
Dysthymia	7	35					
GAD	2	10					
SAD	1	5					
Anxiety disorder NOS	1	5					
Adjustment disorder with dep./anx.	2	10					
Identity problems with dep./anx.	1	5					
	Mean	**SD**	**Mean**	**SD**	**t**	**df**	***p***
CDI: total score[a]	17.58	9.70	4.56	3.51	-6.10	41	<.001
RCADS: total score anxiety subscales[a]	33.23	15.22	14.25	10.94	-4.76	41	<.001
Session 2							
	Mean	**SD**	**Mean**	**SD**	**t**	**df**	***p***
CDI: total score	12.60	9.55	4.20	3.55	-3.99	42	<.001
RCADS: total score anxiety subscales	25.71	15.60	10.83	8.78	-3.98	42	<.001

a=questionnaire data was missing for one participant from the dep/anx group; IQ = Intelligence Quotient, GAD = Generalized Anxiety Disorder, SAD = Social Anxiety Disorder, NOS = Not Otherwise Specified, CDI = Children's Depression Inventory, RCADS = Revised Children's Anxiety and Depression Scale.

Clinical Assessment

In addition to the clinical assessment as part of the standard intake/interview procedures by a child and adolescent psychiatrist, the child and parent versions of the Anxiety Disorders Interview Schedule (ADIS) (Silverman, & Albano, 1996) was used to obtain DSM-IV-based classifications of anxiety and depressive disorders. Standardized dimensional measures were used for assessing the severity of self-reported symptoms of depression and anxiety; i.e. the Children's Depression Inventory (CDI) (Kovacs, 1992) and the Revised Child Anxiety and Depression Scale (RCADS) (Chorpita et al., 2000). The same measures were assessed in the control group, and control participants were excluded if they met criteria for a DSM-IV diagnosis based on the ADIS-interviews or had (sub)clinical scores on clinical questionnaires. Total scores of the CDI and a total score of the five RCADS anxiety scales for both groups were subsequently used in the analyses.

Image Acquisition

Data were acquired using a 3.0T Philips Achieva (Philips, Best, The Netherlands) scanner at the Leiden University Medical Centre. Scanning procedures were described previously (Pannekoek et al., 2014a). RSFC data was acquired at the beginning of the scan sessions. In short, resting-state functional MRI data were acquired for each subject using T2*-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 x 2.75 mm; slice thickness 2.72 mm; total duration of the resting-state run 6 minutes. For the resting-state scan, participants were instructed to lie still with their eyes closed and not to fall asleep. Wakefulness during acquisition was confirmed after the scan. A sagittal 3-dimensional gradient-echo T1-weighted image was acquired for registration purposes with the following scan parameters: TR=9.8 ms.; TE=4.6 ms.; flip angle=8°; 192x152 matrix; FOV=224x177x168 mm, 140 sagittal slices; no slice gap; 1.16x1.16x1.20

mm voxels. Finally, we acquired a high resolution EPI scan for registration purposes with the following scan parameters: TR=2200 ms.; TE=30 ms.; flip angle=80°; 112x109 matrix; FOV=220x220x168 mm, 84 sagittal slices; no slice gap; 1.96x1.96x2 mm voxels. Prior to scanning, all participants were introduced to the scanning situation by lying in a dummy scanner and hearing scanner sounds.

Preprocessing

All data were preprocessed and analyzed using the Oxford Centre for Functional MRI of the Brain (FMRIB) software library version 5.0.4 (FSL; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) (Smith et al., 2004). Preprocessing consisted of nonbrain-tissue removal, motion correction (McFlirt) (Jenkinson, Bannister, Brady, & Smith, 2002), grand mean-based intensity normalization of the entire 4-D data set by a single scaling factor, slice timing correction, spatial smoothing with a 6 mm full width at half maximum Gaussian kernel, and temporal band pass filtering at $0.009 < f < 0.15$ Hz, which improves BOLD signal estimation and produces connectivity patterns that relate most closely to task-based activations (Biswal et al., 1995; Fox, & Raichle, 2007; Fransson, 2006; Roy et al., 2009; Toro, Fox, & Paus, 2008). Finally, the high resolution EPI images were registered to the T1-weighted anatomical images. Thereafter the T1-weighted anatomical images were registered to the 2-mm Montreal Neurological Institute (MNI) standard space image (T1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada). Subsequently, all registrations were combined and used to transform the resting-state (RS) data to MNI space. The maximum allowable displacement due to excessive head motion was set at 3 mm translation or 3º rotation in any direction. To guard against the effects of in-scanner micro-motion on connectivity patterns we implemented motion-censoring (i.e. spike regression) (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012; Satterthwaite et al., 2013). We used FSL's motion outliers tool to detect time points (i.e. frames) in an fMRI dataset that have been corrupted by motion (i.e. spikes; http://

fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLMotionOutliers). Steps included motion correction of individual participant's functional data, calculating framewise displacement (FD) for each time point, thresholding FD at 0.35 (~0.35 mm), and generating a confound matrix to be used in the subject-level general linear model (GLM). By including the confound matrix, spikes were treated as regressors.

Statistical analyses

Self-reported symptomatology

To investigate group differences in time related changes in self-reported symptomatology we used time (2 levels) x group (2 levels) repeated measurement ANOVA's in SPSS 19 (SPSS Inc., Chicago, IL). When sphericity could not be assumed, a Greenhouse-Geisser correction (GG-corr.) was used. All post-hoc comparisons were Bonferroni corrected for multiple comparisons. Values deviating more than three standard deviations from the mean were considered outliers and removed from the analyses (N=1 CLIN for depression questionnaire). Furthermore, expectation maximization was used when a limited amount of items in the CDI (6 items in total), the RCADS (6 items in total) were missing.

Resting-state functional connectivity

We used a seed based approach to study RSFC. Based on previous research, we a priori selected the bilateral amygdala to study connectivity within the limbic network (Pannekoek et al., 2014a). We created a mask in standard space for the amygdala based on the Harvard-Oxford Subcortical Structural Probability Atlas in FSL (Veer, Oei, Spinhoven, Van Buchem, Elzinga, & Rombouts, 2011) (left amygdala 98% probability; right amygdala 94% probability; MNI coordinates ±22, -6, -16; 4 mm. sphere). We also created subject specific masks for white matter (WM) and cerebral spinal fluid (CSF) using FSL's FAST (FMRIB's Automated Segmentation Tool). To prevent partial voluming effects with grey matter, the masks were thresholded at 80%

and subsequently eroded. By including a mask for WM and CSF physiological noise is effectively removed from resting-state data and this approach is favored above global signal regression, which has been shown to distort connectivity patterns (Saad et al., 2012; Weissenbacher, Kasess, Gerstl, Lanzenberger, Moser, & Windischberger, 2009).

The masks (left and right amygdala, WM and CSF) were transformed to functional native space by applying the inverse transformation matrices obtained from the registration procedure, and spatially averaged time series were extracted for each mask and for each subject. For each subject on each occasion 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 using the left and right amygdala seed masks were entered as regressors in a GLM. To correct for physiological and motion-related noise, the time courses of both the WM and CSF masks were added to all analyses as confound regressors along with six motion parameters (three translations and three rotations) and parameters obtained from the motion censoring procedure.

After reslicing the resulting parameter estimate maps and their corresponding within-subject variance maps into 2 mm isotropic MNI space, they were entered into higher-level analyses. Three different higher level analyses were performed. The first comprised of a 2-way mixed effect ANOVA, examining the group x time interaction. The second and third analysis comprised of a higher level within and between groups mixed effects analysis (one- and two-sample t-tests), one for each session. In all analyses the number of framewise displacements were entered as a confound regressor. The one- and two-sample t-tests for session 1 and session 2 also included age at session 1 and gender as confound regressors. Since structural studies have indicated structural abnormalities in childhood anxiety and depression (Hulvershorn et al., 2011; Pannekoek et al., 2014b; Pine, 2007) we used gray matter density information of each subject as a voxel-dependent confound regressor in all our analyses. To correct for multiple comparisons (on whole

brain level), cluster correction was applied in all group analyses with significance set at a corrected $p<.05$ and an initial cluster-forming threshold of $Z>2.3$.

Relation between RSFC and self-reported symptomatology:

To further examine the relation between longitudinal changes in RSFC and longitudinal changes in self-reported depression and anxiety symptomatology, we calculated individual mean z-scores for connectivity based on the session x group interaction using Featquery, as implemented in FSL (Smith et al., 2004). Thereafter, we computed difference scores for connectivity values, self-reported depression symptoms and self-reported anxiety symptoms by subtracting the value at session 2 from the value at session 1 using SPSS 19 (SPSS Inc., Chicago, IL). Finally, partial correlation analyses were performed including age at session 1 and gender as covariates.

Results

Self-reported depression and anxiety symptoms

The repeated measurement ANOVA for CDI total score resulted in a main effect for session ($F_{(1,40)}=9.38$, $p<.005$), a main effect for group ($F_{(1,40)}=28.99$, $p<.001$) and a session x group interaction effect ($F_{(1,40)}=6.98$, $p<.05$). At both sessions the clinical group reported significantly more depression symptoms than the control group (both p's$\leq$.001) and only the clinical group showed a significant decrease in symptomatology (p=.001). There was no change for the control group.

The repeated measurement ANOVA for the total anxiety scale of the RCADS resulted in a main effect of session ($F_{(1,41)}=10.27$, $p<.005$) and a main effect of group ($F_{(1,41)}=23.12$, $p<.001$), but no session x group interaction. Self-reported anxiety scores were higher at session 1 compared to session 2 and the clinical group reported significantly more anxiety symptoms than the control group (see Figure 1).

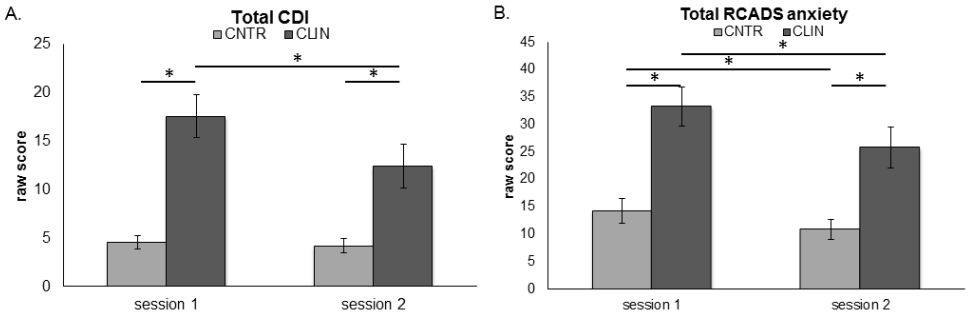

Figure 1. Overview of longitudinal changes in (a) self-reported depression symptoms (CDI) and (b) self-reported anxiety symptoms (RCADS). ** p < 0.05; CNTR = control group; CLIN. = clinical group.*

RSFC in depressed and anxious adolescents

The one-sample t-test analyses for left amygdala, right amygdala and bilateral amygdala connectivity in both groups resulted in significant positive connectivity with subcortical and cortical regions, including hippocampus, parahippocampal gyrus, thalamus, putamen, medial PFC, inferior frontal gyrus, orbitofrontal cortex (OFC), frontal pole and temporal pole at both time points (Figure 2). These connectivity patterns correspond to patterns reported previously (Pannekoek et al., 2014a). Furthermore, at session 2 the control group showed significant negative connectivity between the left amygdala and the right middle frontal gyrus and from the right amygdala to the left frontal pole.

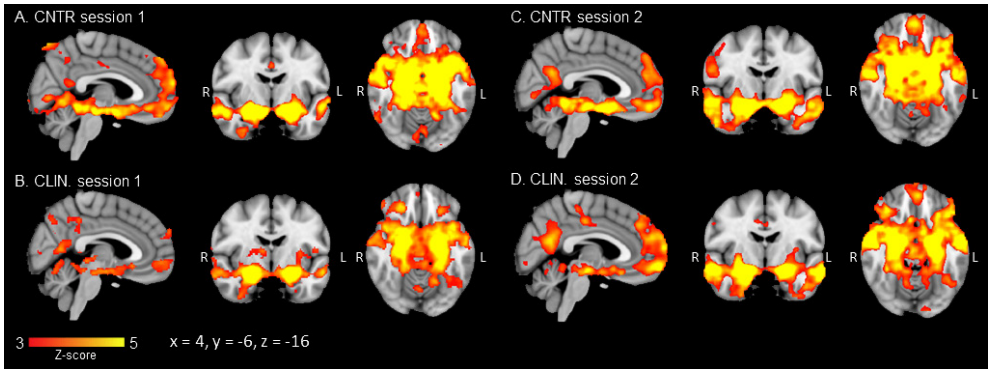

Figure 2. Positive bilateral amygdala connectivity for (a) the control group at session 1, (b) the clinical group at session 1, (c) the control group at session 2 and (d) the clinical group at session 2. *Bilateral amygdala shows positive connectivity with several subcortical and cortical regions. Images are thresholded z-statistic, overlaid on the MNI-152 standard brain. Yellow to red are z-values, ranging from 3 to 5. CNTR = control group; CLIN. = clinical group.*

Longitudinal changes in RSFC

Figure 3a displays the seeds of left and right amygdala, which were submitted to the ANOVA. The 2-way mixed effect ANOVA showed both session and session x group effects. The session x group interaction resulted in significant changes in connectivity between right amygdala and the dorsomedial prefrontal cortex (DMPFC), specifically the left superior frontal gyrus/ right paracingulate gyrus (see Figure 3b).

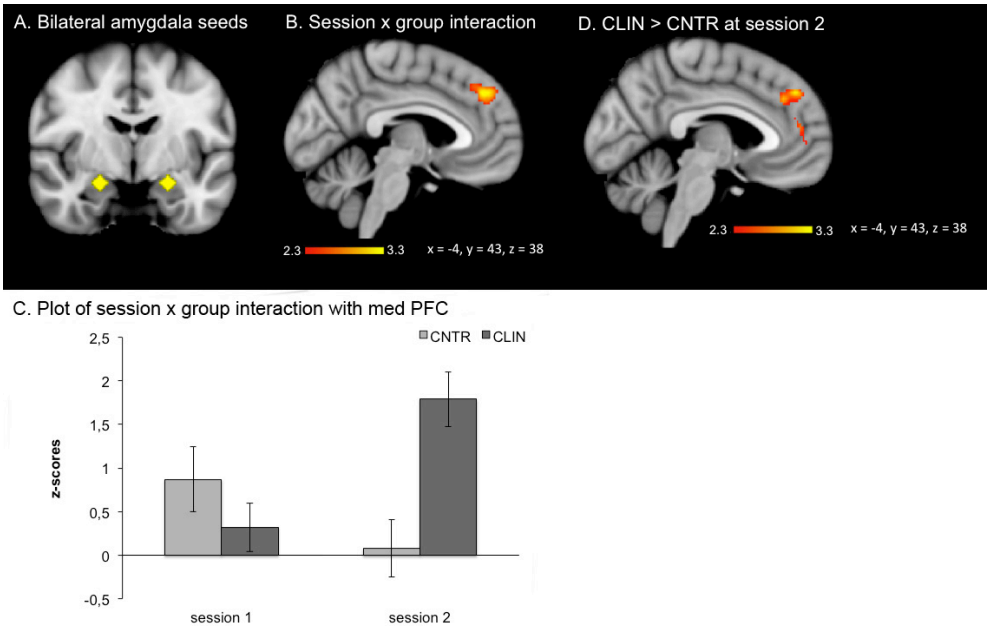

Figure 3. Overview of time related changes in functional connectivity from the amygdala: *(a) bilateral amygdala seeds (central voxel: ±22, -6, -16; 4 mm sphere), (b) significant positive connectivity between right amygdala and medial PFC as revealed by a session x group interaction, (c) z-values for right amygdala – med PFC connectivity separately for each group and each session, and (d) significantly more positive connectivity between right amygdala and medial PFC within the clinical group compared to the control group at session 2 as revealed by a two-sample t-test. Brain images are thresholded z-statistics, overlaid on the MNI-152 standard brain. Yellow to red are z-values, ranging from 2.3 to 3.3. CNTR = control group; CLIN. = clinical group.*

To further examine the session x group effect, individual z-scores for this connectivity pattern were calculated. The results of this analysis indicated an increase in positive connectivity between right amygdala and DMPFC

for the clinical group (see Figure 3c). This effect was further supported by the results of the two-sample t-tests: At session 1 there was no significant group difference in connectivity between these regions, while at session 2 the clinical group showed more positive connectivity between right amygdala and DMPFC compared to the control group (see Figure 3d). As can be seen in Figure 3C, there was no significant increase or decrease in connectivity for the control group.

Relation with changes in self-reported symptomatology

To examine the relation between longitudinal changes in RSFC between right amygdala and DMPFC, and changes in self-reported symptomatology, we correlated difference scores of connectivity, depression and anxiety symptomatology across groups and within the clinical group separately. For the combined group (N=42), we found a significant negative correlation ($r=-.34$, $p < .05$) between change in connectivity and change in self-reported depression symptomatology, with participants showing a larger increase in right amygdala - DMPFC connectivity also showing a larger decrease in self-reported depression symptoms (see Figure 4). The correlations were not significant when examining the groups separately (clinical $p<.60$, control group $p<.25$), showing that the effects were found across all participants. No significant correlations were found for changes in anxiety symptomatology.

Discussion

The goal of this study was to examine longitudinal changes in RSFC in treatment naïve adolescents with depressive and anxiety disorders. We also examined whether changes in RSFC relate to changes in self-reported depression and anxiety symptomatology. Previous research based on cross-sectional assessments indicated significant differences in RSFC between adolescents with depressive and anxiety disorders and healthy control adolescents, specifically in relation to the amygdala (Mcclure et al., 2007b; Monk et

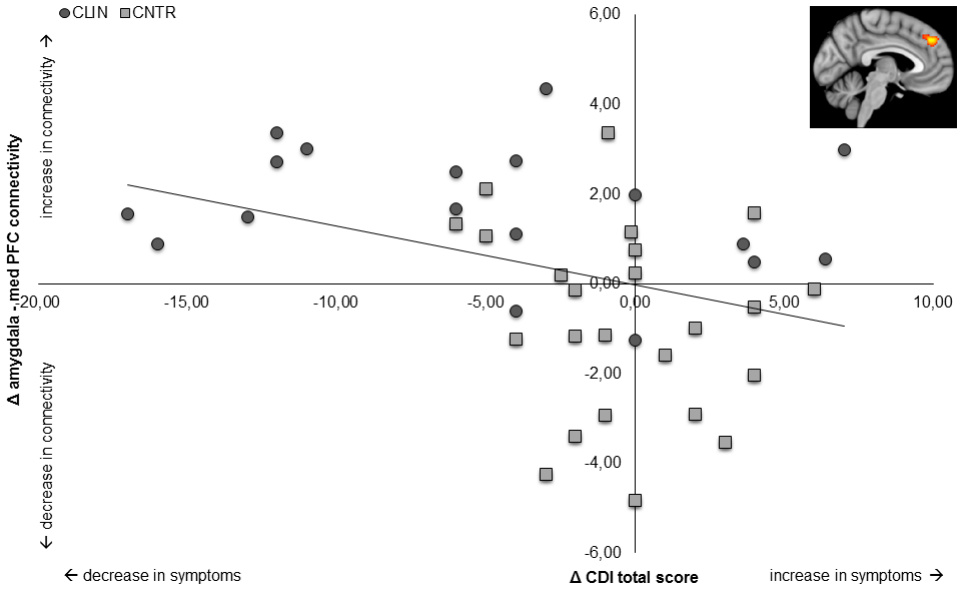

Figure 4. Negative correlation between change in right amygdala – medial PFC connectivity and change in self-reported depression symptoms. *Positive values on x-axis indicate an increase in depression symptoms over time while negative values indicate a decrease in depression symptoms. For the y-axis, positive values indicate an increase in right amygdala – medial PFC connectivity while negative values indicate a decrease in connectivity. CNTR = control group; CLIN. = clinical group.*

al., 2008a; Roberson-Nay et al., 2006; Thomas et al., 2001a). The results of the current study showed a significant decrease in self-reported depression and anxiety symptoms and a significant increase in positive connectivity between right amygdala and medial PFC in adolescents with anxiety and depression using longitudinal analyses. Furthermore, changes in right amygdala - DMPFC connectivity were related to changes in self-reported depression symptomatology within the complete sample, such that stronger connectivity increase was associated with a larger decrease in symptoms.

First, we examined whether the effects of the resting state analyses were comparable to prior research (Jin et al., 2011; Pannekoek et al., 2014a). Indeed, at both sessions both groups showed strong positive connectivity patterns between bilateral amygdala and other subcortical and cortical re-

gions, including medial PFC, bilateral orbitofrontal cortex (OFC), bilateral frontal pole and bilateral temporal pole. It is suggested that the connectivity between these regions is important for emotion processing and regulation (Grecucci, Giorgetta, Bonini, & Sanfey, 2013) and therefore are important brain regions to focus on when examining adolescents with depressive and anxiety disorders. Contrary to our expectations, we were not able to replicate the group differences at session 1 previously reported by our group (Pannekoek et al., 2014a). This might be due to a difference in the composition of the groups and the different approach for correcting for motion outliers/ spiking and therefore are important target regions for depression and anxiety and possibly also for treatment outcome.

The main question of this study concerned longitudinal changes in connectivity patterns in adolescents who received CBT-based treatment for anxiety and depression. The longitudinal comparison revealed a significant interaction between session and group in which the clinical group showed increased positive connectivity between right amygdala and DMPFC over time. The control group did not show a significant increase or decrease in connectivity. Based on the existing literature there might be a plausible interpretation for the effects. It is possible that after treatment DMPFC exerts stronger top down control over the amygdala. This line of reasoning fits well with general ideas about depression and anxiety in which it is proposed that extended reactivity of the amygdala is not effectively regulated by the medial PFC (Mayberg, 1997). Treatment for depression and anxiety (e.g. exposure therapy or cognitive behavioral therapy (CBT)) might target this effect by increasing top-down control of emotional processes (Quide et al., 2012). This in turn might increase the functional connectivity between amygdala and medial PFC.

Longitudinal research in adults with depressive or anxiety disorders indeed showed an increase in PFC activation in relation to CBT, which might reduce amygdala reactivity (Clark, & Beck, 2010; Fu et al., 2008; Månsson et al., 2013). This interpretation finds further support in the significant cor-

relation between changes in self-reported depression scores and change in connectivity between right amygdala and medial PFC: adolescents who show a larger increase in positive connectivity also show a larger decrease in depression symptoms. That is to say, adolescents who report to feel better over time also show a more positive connectivity over time. This effect was only found when both groups were combined. In the clinical group there was no significant relation between change in connectivity and change in self-reported symptomatology. Possibly, this can be explained by a lack of power: a larger sample would have allowed us to better investigate individual differences in depression and anxiety symptomatology and the relation with longitudinal changes in RSFC. Another explanation might be that CBT-based treatment has a larger effect on the neuronal level than on the symptom level.

Recent studies suggest that adolescence might be a special period in which treatment of depression and/or anxiety symptoms results in increased sensitivity of the amygdala instead of increased PFC regulation, which is typically found in adult studies (Drysdale et al., 2013; Maslowsky et al., 2010; Pattwell et al., 2012). Therefore, future research is necessary to investigate the specificity of increased amygdala activation after receiving treatment by using task related fMRI and RSFC. It would be of interest to perform a large longitudinal study in which depressed and anxious adolescents are included who are referred for different forms of treatment e.g. structured CBT procedures and medication. This will provide the opportunity to examine the influence of individual differences in depression and anxiety symptomatology and eliminates inter individual treatment effects.

Although the results of the current study are very interesting and provide new insights on longitudinal changes in RSFC in adolescents with depressive and anxiety disorders, there are some limitations. First, we used a combined sample of adolescents with depressive and/or anxiety disorders. Comorbidity and overlap in symptomatology between depression and anxiety is high, especially during adolescence (Essau, 2003). Therefore, we included a combined sample to make the study representative for clinical

practice. However, this did not allowed us to evaluate the specific contribution of changes in depressive and anxiety symptoms on changes in RSFC. Possibly, individual changes in depression symptoms are more related to changes in RSFC than individual differences in anxiety symptoms, as the correlation analysis in our study suggests. Second, the current sample included more females than males. Even though the numbers of females and males were equal between groups and we controlled for gender in all analyses, it is possible that the effects reported are mainly generalizable to females and not to males. The unequal distribution of females and males however, is representative for clinical practice: It is well known that there are many more females with a depressive or anxiety disorder than there are males. Finally, we allowed stable use of SSRI's within the clinical group (N=2). To make sure these two participants did not drive the interaction effect, we also evaluated the results when excluding the participants that used SSRI's. The results did not change. Future studies should try to take these limitations into account when performing a longitudinal study.

Taken together, this study highlights the importance of studying differentiating patterns of resting state functional connectivity in adolescents with depressive and/or anxiety disorders. The longitudinal design enabled us to examine individual trajectories of both RSFC and self-reported symptomatology. The results of our study revealed significant changes in amygdala – medial PFC connectivity in adolescents with depressive and anxiety disorders who received CBT-based treatment. Although the directionality of the connectivity effects is not interpretable, we have discussed a potential hypothesis. Future research should further investigate longitudinal changes in both RSFC and task-related activation, specifically in adolescence since an increasing number of studies indicate that adolescence is a dynamic period in terms of brain and behavioral development (Casey et al., 2008; Gogtay et al., 2004; Somerville, & Casey, 2010). By performing more longitudinal studies, we might eventually be able to predict which children will develop depressive and anxiety disorders and improve treatment and intervention strategies.