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

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Chapter 5

Longitudinal changes
in amygdala activation

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van den Bulk, B.G., Cousijn, J., van Lang, N.D.J., van der Wee, N.J.A., Rombouts, S.A.R.B., Crone, E.A., Vermeiren, R.R.J.M. Amygdala reactivity to emotional faces in depressed and anxious adolescents: a longitudinal fMRI study across treatment.

Abstract

Cross-sectional fMRI studies showed abnormal amygdala reactivity in response to emotional faces in adolescents with depressive and anxiety disorders. Little is known about how amygdala reactivity changes over the course of treatment and how this relates to individual differences in symptom severity during adolescence. In this longitudinal fMRI study, 19 treatment-naïve adolescents with a DSM-IV depressive and/or anxiety disorder and 23 healthy adolescents were scanned three times in a 6-month period. The clinical group received treatment as usual (CBT-based) in-between scan sessions. Brain activity in the amygdala and dorsolateral prefrontal cortex (DLPFC) was recorded during an emotional face-processing task with fearful, happy and neutral faces and compared between groups over time. Symptoms of depression and anxiety and self-reported stress levels significantly decreased in the clinical group over time. A significant session x group interaction indicated higher left amygdala activity in the clinical group compared to the control group at the third session only, regardless of whether the face depicted a fearful, happy or neutral emotion. For DLPFC, there were no significant session or group (interaction) effects. The results of this study point to an increased sensitivity to emotional faces in adolescents with depressive and/or anxiety disorders receiving CBT-based therapy, which possibly indicates different treatment-related neural changes in adolescents compared to adults. These findings highlight the importance of taking adolescent brain development into account when trying to unravel the neurobiological mechanisms underlying treatment responsiveness.

Introduction

Depressive and anxiety disorders are highly prevalent, have long-term effects on psychological well-being and often emerge during adolescence (Costello et al., 2011; Kessler et al., 2012b). Co-occurrence of depressive and anxiety disorders is high (Essau, 2008) and increases the risk for a more negative outcome compared to having only one disorder (Lewinsohn et al., 1995). Gaining deeper insight into the onset and course of depressive and anxiety disorders is needed, as a substantial part continues to suffer consequences over the long run (In-Albon, & Schneider, 2007). Unraveling the underlying neurobiological mechanisms therefore is an important step in the development and optimization of treatment.

Previous cross-sectional neuroimaging studies indicated that depressive and anxiety disorders are associated with heightened amygdala activation during processing of emotional faces (anxiety (Mcclure et al., 2007b; Monk et al., 2008b); depression (Monk et al., 2008a; Perlman et al., 2012; Roberson-Nay et al., 2006)), co-varying with self-reported levels of anxiety and heightened amygdala activation (Thomas et al., 2001a; Van Den Bulk et al., 2014). Heightened amygdala activation seems to be related to a disturbed top-down regulation, in which the amygdala is not effectively controlled by prefrontal cortex regions (PFC) (Blair et al., 2012; Johnstone, Van Reekum, Urry, Kalin, & Davidson, 2007). Cognitive Behavioral Therapy (CBT) may be effective in the treatment of depression and anxiety by increasing top-down control of emotional processes (Quide, Witteveen, El-Hage, Veltman, & Olff, 2012). An important question concerns how CBT-based therapy influences brain functioning. Prospective studies in depressed adults indicated that heightened patterns of pre-treatment amygdala activation are predictive for better treatment outcome (Canli et al., 2005; Siegle, Carter, & Thase, 2006). One of these studies (Siegle et al., 2006) also reported that depressed adults with low levels of sub-genual anterior cingulate cortex activation showed higher levels of improvement after CBT. A longitudinal study in 10-16 year old adolescents with anxiety disorders replicated the effects on amygdala acti-

vation (Mcclure et al., 2007a). However, this study did not include a healthy control group, which makes it hard to interpret these results in the light of normal development. Longitudinal studies in adults with depressive or anxiety disorders (Fu et al., 2008; Månsson et al., 2013) showed that CBT is associated with increases in activity in PFC areas and thereby possibly reduces amygdala activation (Clark, & Beck, 2010; Quide et al., 2012), but it remains unclear whether these changes are also present in adolescents.

An important consideration when examining the neural responses of the amygdala and PFC in adolescents in relation to depression and anxiety is that typical maturation during adolescence is marked by an increase in reward sensitivity with relatively heightened amygdala activity in mid adolescence in response to emotional faces (Casey et al., 2011; Hare et al., 2008; Pfeifer et al., 2011). Furthermore, in children (4-9 years of age) amygdala and PFC are often active together, whereas in adults (18-22 years of age) heightened PFC activity is found in combination with reduced amygdala activity (Gee et al., 2013b). Finally, in contrast to adult studies, Maslowsky and colleagues reported increases in amygdala and ventral lateral PFC activation over an 8-week period in a sample of 7 adolescents with generalized anxiety disorders referred for CBT compared to controls (Maslowsky et al., 2010). Together, these studies suggest that CBT may have a different effect on brain activity related to emotional face processing in adolescents and in adults, warranting further longitudinal studies investigating the effect of CBT on brain activity over the course of treatment in adolescents.

The goal of the current study therefore was to investigate time related changes in amygdala and PFC activation to emotional faces in treatment naïve adolescents with a depressive and/or anxiety disorder and healthy age-matched control participants. We scanned 30 adolescents with depressive and anxiety disorders and 31 age-matched controls at intake, after 3 months and after 6 months. The clinical group received CBT-based treatment in-between the first and last session. We tested whether repeated exposure to emotional faces would result in less habituation (Hare et al., 2008) or ele-

vated sensitivity to emotional faces (Maslowsky et al., 2010) in clinical adolescents, or dampened sensitivity, similar to what has been found in studies with clinical adults (Clark, & Beck, 2010; Quide et al., 2012). Concerning PFC activation, we examined comparable hypothesis and expected to find an increase in PFC activation over time in the clinical group (Maslowsky et al., 2010). Furthermore, we tested whether change in amygdala and PFC reactivity was related to a change in self-reported symptoms of depression and anxiety (Thomas et al., 2001a).

Methods

Participants

The original study sample consisted of 61 participants at session 1 (Van Den Bulk et al., 2014), of which 19 were excluded for the current analyses due to various reasons. At session 1, seven participants (N=4 clinical; N=3 control) were excluded due to technical scanning problems, unforeseen clinical features or anomalous findings reported by the radiologist. At session 2 and session 3, 12 additional participants (N=7 clinical; N=5 control) were excluded because of technical problems during scanning, contra indications for fMRI, excessive head movement (round off >4 mm.), or dropped out of the study because they were no longer interested or eligible (complex family problems, compulsory admission, broken contact).

The final sample consisted of 19 treatment-naïve adolescents with a clinical diagnosis of a current DSM-IV depressive or anxiety disorder and 23 healthy controls that completed 3 functional Magnetic Resonance Imaging (fMRI) sessions. FMRI data for the clinical group were collected before the start of regular CBT (session 1), and three (session 2) and six months (session 3) after session 1. The adolescents in the control group were scanned within the same time interval without receiving treatment. There were no significant differences between the groups considering age and sex (Table 1).

Adolescents from the clinical group were recruited in outpatient de-

partments of two child and adolescent psychiatric institutes in Leiden. They were diagnosed with any DSM-IV depressive or anxiety disorder and referred for cognitive behavioral therapy (CBT). 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.

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	19		23				
Females/Males	18/1		19/4		1.46	1	.36
	Mean	**SD**	**Mean**	**SD**	**t**	**df**	***p***
Age session 1	15.78	1.50	15.11	1.44	-1.47	42	.15
Full scale IQ	106	8.40	107	7.50	.42	42	.68
Weeks between sessions							
Session 1 – Session 2	14.21	1.58	14.17	1.67	-.072	40	.94
Session 2 – Session 3	14.37	1.74	14.13	1.84	-.427	40	.67
Session 1							
DSM-IV Classification:	**N**	**%**	**N**	**%**			
No disorders	0	0	26	100			
Depression	6	35.58					
Dysthymia	8	42.11					
GAD	2	10.53					
SAD	1	5.26					
Adjustment disorder with dep./anx.	2	10.53					
	Mean	**SD**	**Mean**	**SD**	**t**	**df**	***p***
CDI: total score	18.20*	9.39	4.11	3.18	-6.74	39	<.001
RCADS: total score anxiety subscales	33.68*	14.79	14.00	11.11	-4.88	39	<.001
Session 3							
	Mean	**SD**	**Mean**	**SD**	**t**	**df**	***p***
CDI: total score	12.47	9.22	3.74	3.41	-4.22	40	<.001
RCADS: total score anxiety subscales	24.22	14.43	10.56	9.09	-.373	40	.001

*=questionnaire data was missing for one participant; 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.

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 Centre approved the study and all anatomical scans were reviewed and cleared by a radiologist.

Clinical Assessment and CBT treatment

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 a 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). Total scores of the CDI and the RCADS-anxiety scale (sum of five anxiety subscales) were subsequently used in the analyses. The same measures were assessed in the control group, and control participants were excluded if they met the criteria for a DSM-IV diagnosis based on the ADIS-interviews or had (sub)clinical scores on clinical questionnaires. All adolescents in the clinical group received CBT-based treatment within the clinical setting (treatment as usual). Treatment was administered by registered and trained clinicians (psychologists/psychiatrists). The duration of treatment and the number of sessions varied between participants. For most participants, treatment lasted the entire six months.

Task

At each session we administered an emotional face-processing task (Van Den Bulk et al., 2013; Van Den Bulk et al., 2014). In short, the task consisted of three randomly presented constrained (state questions: 'how afraid are you?', 'how happy are you?' and 'how wide is the nose?') and one unconstrained (passive viewing) state conditions. After state presentation, participants viewed 21 pictures expressing a fearful, neutral or happy face (a total of 21 trials per state condition; presented in random order), which they had to rate on a four-point rating scale (1. not at all, 2. a little, 3. quite and 4. very). Reaction times and subjective scoring of the different emotional faces (fearful, happy or neutral) were recorded for behavioral analyses.

All trials had the same structure: first participants were presented with one of the state questions for 4000 milliseconds followed by a fixation cross with a jittered duration between 500 and 6000 milliseconds. Thereafter, one of the pictures was shown for 3000 milliseconds during which participants had to rate the pictures (Figure 1). Trials during which the participants did not respond within 3000 milliseconds (1.38% in total across all sessions) were not included in the behavioral analyses and were included as a covariate of no interest in the fMRI analyses. Self-reported stress levels were measured just after the start, in the middle and near the end of each scan session with the use of a Visual Analogue Scale (VAS) ranging from 0-100.

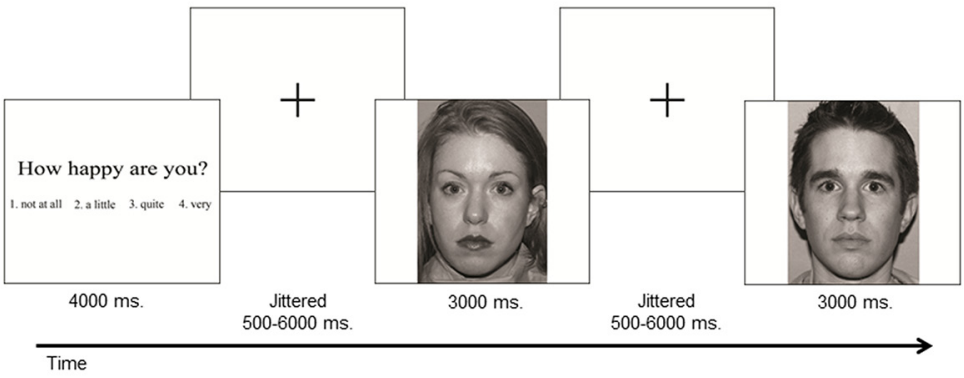

Figure 1. Emotional face-processing task. *Participants were presented with one of the state questions (i.e., how happy are you, how afraid are you, how wide is the nose or passive viewing) followed by a fixation cross. Thereafter, one picture of a negative, positive or neutral face was shown during which participants had to rate the pictures (1=not at all, 4=very).*

Image Acquisition

Data were acquired using a 3.0T Philips Achieva (Philips, Best, The Netherlands) scanner at the Leiden University Medical Centre. Stimuli were presented onto a screen located at the head of the scanner bore and viewed by the participants with a mirror mounted to the head coil assembly. T2*-weighted Echo-Planar Images (EPI) (TR=2200 ms., TE=30ms, flip angle=80°, 80x80 matrix, FOV=220 mm, 38 slices of thickness 2.72 mm) were obtained during three functional runs of 192 volumes each. For each run, the first two volumes were discarded to allow for equilibration of T1 saturation effects. Also, 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.

fMRI analyses

We used SPM8 (Welcome Department of Cognitive Neurology, London) to analyze the acquired data. Data was preprocessed using the following steps: realignment of functional time series to compensate for small head movements and differences in slice timing acquisition, registration and normalization of functional volumes (from EPI to individual structural T1 and thereafter to the T1 template) and spatially smoothing the functional volumes with an 8mm, full-width at half-maximum isotropic Gaussian kernel. The normalization algorithm used a 12-parameter affine transformation together with a nonlinear transformation involving cosine basis functions and resampled the volumes to three mm. cubic voxels. The MNI (Montreal Neurological Institute) 305 stereotaxic space templates (Cocosco et al., 1997) were used for visualization and all results are reported in this template, which is an approximation of Talairach space (Talairach, & Tournoux, 1988).

Individual subjects' data (per participant and per session) were analyzed using the general linear model in SPM8. The fMRI time series for each emotional face in each state condition (a total of 12 conditions) was modeled

as a zero duration event convolved with a canonical hemodynamic response function (HRF). The presentations of state questions were modeled separately as 4000 millisecond events and were added as covariates of no interest. The modeled events were used as a covariate in a general linear model along with a basic set of cosine functions that high-pass filtered the data. The least squares parameter estimates of the height of the best-fitting canonical HRF for each condition were used in pair wise contrasts. The resulting contrast images, computed on a subject-by-subject basis, were submitted to group analyses. At the group level, we performed flexible (main effect of session and interaction effect session x group) and full (task related effects) factorial models. Task- and time-related responses were considered significant if they consisted of at least 10 contiguous voxels at a FDR cluster-corrected threshold of $p<.05$ (see Supplement 1). These findings are reported in the supplementary material.

In the current study, we used a priori ROI selection to test our hypotheses about changes in amygdala and dorsolateral PFC (DLPFC) reactivity, both commonly activated during emotional face processing (Fusar-Poli et al., 2009). We used the MarsBaR toolbox implemented in SPM8 (http://marsbar.sourceforge.net/) (Brett et al., 2002) to extract percent signal change in the amygdala (anatomically defined with the AAL atlas) and DLPFC (functionally defined and masked with the AAL atlas template for mid frontal) for each condition. The percent signal change values were further analyzed using repeated measurement ANOVAs in SPSS 19 and all post-hoc tests were Bonferroni corrected for multiple comparisons.

Analyses plan

Scores on self-reported stress levels, questionnaires, reaction times and subjective ratings of the stimuli during the face-processing task were examined over sessions and between groups using repeated measurement ANOVAs in SPSS 19. Analyses of the reaction times and subjective ratings of the emotional faces can be found in Figure S1.

A series of stepwise regression analyses were performed to investigate if the change in amygdala reactivity over time in the clinical group was related to 1) baseline symptom severity, 2) change in symptom severity, 3) number of treatment sessions and 4) change in self-reported stress levels. In all these analyses, mean percent signal change in the amygdala at session 3 was the dependent variable, and mean percent signal change at session 1 and session 2 were entered as independent variables in step one, with either the RCADS-anxiety and CDI scores, change in RCADS-anxiety and CDI scores, number of treatment sessions, or change in self-reported stress levels as independent variables in step 2.

Results

Stress level and symptoms of depression and anxiety over time

For self-reported stress levels we found a main effect of session ($F_{(2,68)}$=24.49, p<.001) and a session x group interaction effect ($F_{(2,68)}$=3.73, p<.05). The clinical group showed significantly higher stress levels at session 1 compared to the control group (p<.05) and there was a significant decline in stress levels between session 1 and 2 and between session 2 and 3 (p's<.05). For the control group, no change was observed over time (session 1–session 2 p=.148 and session 2–session 3 p=.753).

For the CDI total scale, we found a main effect of session ($F_{(2,78)}$=13.65, p<.001, GG-corr.), a main effect of group ($F_{(1,39)}$=37.15, p<.001) and an interaction effect between session x group ($F_{(2,78)}$=8.29, p<.005, GG-corr.). Overall, the clinical group reported significantly higher levels of depression symptoms than the control group (p<.001) and there was a significant reduction in symptom severity between session 1 and session 2 and between session 2 and session 3 (p's<.001) within the clinical group, but not within the control group (p=.805 and p=.528).

For the RCADS-anxiety scale we found a main effect of session ($F_{(2,76)}$=13.03, p<.001, GG-corr.) and a main effect of group ($F_{(1,38)}$=20.63,

p<.001). There was a significant decrease in self-reported anxiety symptoms between session 1 and session 2 and between session 1 and session 3 (p's<.005) across participants, and the clinical group reported significantly higher levels of anxiety compared to the control group (p<.001). See Figure 2 for an overview.

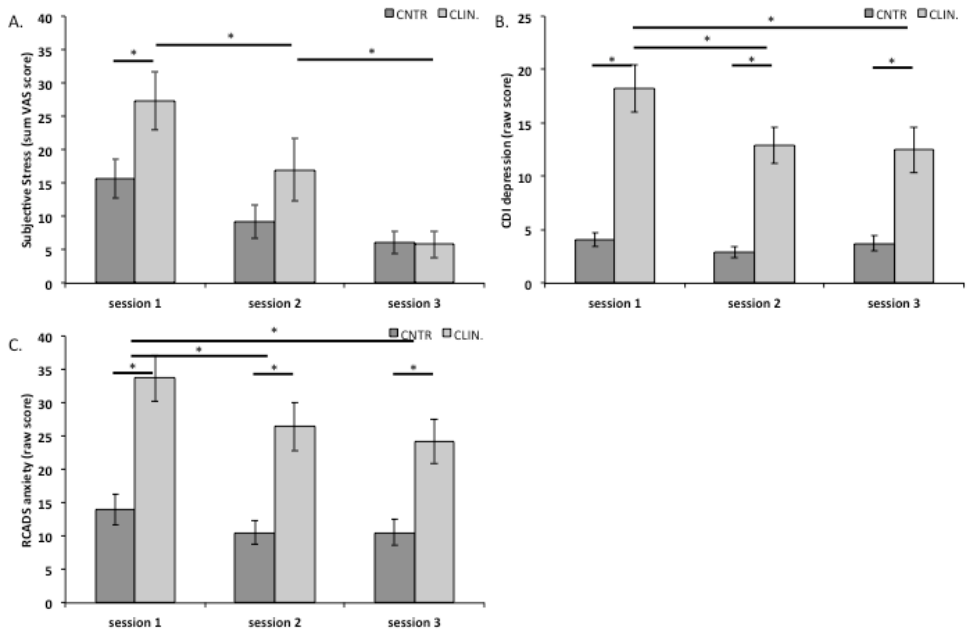

Figure 2. Subjective stress rated on a Visual Analogue Scale (VAS) during scanning (A), symptoms of depression measured with the Children's Depression Inventory (CDI) (B) and symptoms of anxiety over time measured with the anxiety subscale of the Revised Children's Anxiety and Depression Scale (RCADS) (C). ** p < 0.05; CNTR=control group; CLIN.=clinical group.*

Amygdala

The session x state question x emotion x group repeated measurement ANOVA for left amygdala resulted in a main effect of emotion ($F_{(2,80)}$=7.39, p<.005) and an interaction effect between session x group ($F_{(2,80)}$=3.29, p=.042). The main effect of emotion showed that left amygdala was more active for fearful and happy faces than for neutral faces (both p's<.01), while the activi-

ty for fearful and happy faces did not significantly differ (p=1.00). The session x group interaction indicated that the clinical group, compared to the control group, showed more left amygdala activation at session 3 (p=.016) due to an increase in amygdala activation over time in the clinical group (session 1-session 3 p=.069). The same analysis for the right amygdala did not result in effects for session, emotion or group and no interaction effects (see Figure 3). Moreover, there were no main or interaction effects for state question.

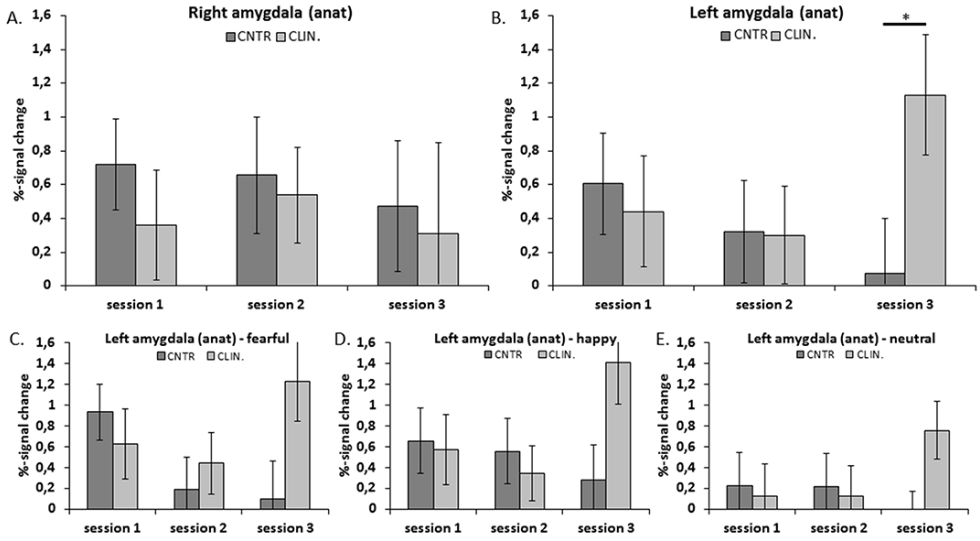

Figure 3. Time related changes in amygdala reactivity in the clinical group and the control group. *(A) Interaction between session and group for left and right amygdala, (B) Interaction between session and group for left amygdala separately for fearful, happy and neutral faces and collapsed across state questions. * p < 0.05; CNTR=control group; CLIN.=clinical group.*

DLPFC

The repeated measurement analysis for left DLPFC indicated a main effect of state question ($F_{(3,120)}$=14.57, p<.001) and emotion ($F_{(2,80)}$=4.87, p=.01). Participants showed more DLPFC activation for active state questions compared to passive viewing (all *p's*<.05) and there was more activation for 'How happy are you?' compared to 'How afraid are you?' (both *p's*<.05). Concerning the emotion effect, participants showed more DLPFC activation for fearful

then for neutral faces (p<.05). The analysis of right DLPFC activations showed a similar main effect of state question ($F_{(3,120)}$=23.09, p<.001) with higher levels of activation for the active state questions compared to the passive viewing condition (all $p's$<.05). Also, activation for 'How happy are you?' and 'How wide is the nose' was higher than for 'How afraid are you?' (both p's<.005). There were no main or interaction effects for group and session (see Figure S2 and Table S1).

Predictors of amygdala activation over time

To further investigate the relation between change in brain activity and change in self-reported symptomatology, we performed a correlation analyses between the significant change in left amygdala activation and several behavioral measures. Left amygdala activity on session 3 was not significantly related to activity at session 1 and session 2. Moreover, neither baseline symptoms of depression (CDI) and anxiety (RCADS) nor change in symptoms of depression and anxiety over time significantly predicted change in amygdala activity. Finally, there was no significant association between change in amygdala activity and number of treatment sessions across time and change in self-reported stress levels over time.

Discussion

Adolescence is a time of major reorganization in brain structure and function (Giedd et al., 1999), which may indicate that special treatment programs are needed for adolescents who experience problems with emotion regulation. We conducted a longitudinal study in which we investigated time related changes in amygdala and DLPFC activation during an emotional face processing task in adolescents with DSM-IV depressive and anxiety disorders compared to a healthy control group. The results showed a significant increase in left amygdala activation over a 6-month period in the clinical adolescents who received CBT-based treatment and this increased sensitivity was found for all depicted emotions (i.e., fearful, happy and neutral faces). DLPFC

activity did not differ between groups and change over time. These findings point to an increasing sensitivity to emotional stimuli in adolescents with high levels of depression and anxiety during treatment, and provides a starting point for understanding this dynamic period in development.

The current findings are in favor of the idea that the amygdala becomes increasingly sensitive with repeated exposure to emotional faces in adolescents with depression and anxiety, whereas no such change was observed in healthy adolescents. A previous study by Maslowsky and colleagues (2010) showed similar results: an increase in amygdala activation after an 8-week period of CBT in a small group of adolescents with a generalized anxiety disorder. Moreover, Hare and colleagues (2008) reported that habituation of the amygdala response to emotional faces is present in adolescents with low levels of trait anxiety, whereas negative values (i.e., suggesting increased sensitivity) were present in adolescents with high levels of trait anxiety. Finally, recent research indicated that adolescents between 12-15 years old show a prolonged process of fear extinction after fear conditioning (Drysdale et al., 2013; Pattwell et al., 2012). This prolonged fear extinction might result in increased sensitization of amygdala reactivity to emotional stimuli.

Since fear extinction is an important component of most CBT-based treatments (Drysdale et al., 2013; Pattwell et al., 2012), CBT may increase amygdala reactivity by sensitizing the adolescents with a depressive and/or anxiety disorder to emotional stimuli. Notably, in the current study increased amygdala reactivity was present independent of the valence of the face, suggesting that the effect could represent a generally increased sensitivity and not necessarily only an increased sensitivity to negative stimuli. Prior developmental models have suggested that adolescence is a period of changes in subcortical and cortical brain regions which may result in increased sensitivity to negative developmental trajectories (e.g. risk taking), while also providing possibilities for the positive effects of treatment (Crone, & Dahl, 2012) . A recent study by Gee and colleagues (2013b) is in line with this hypothesis: their results showed that children often activate the amygdala and

PFC together (positive connectivity) while young adults show heightened PFC activation in combination with decreased amygdala activation (negative connectivity). Future studies should test whether increased sensitivity to emotional faces over time is uniquely associated with CBT treatment or if this is also present in adolescents with anxiety/depression who receive another form of treatment, e.g. medication.

Alternatively, levels of distress in the treatment group may influence amygdala reactivity during a test session, thus influencing baseline amygdala activity. Similarly as in other studies (Clark, & Watson, 1991), we observed a reduction of self-reported stress levels over the course of CBT. The increase in amygdala reactivity at session 3 may then reflect a reduction in baseline amygdala activity due to a reduction in active distress, rather than an increase in amygdala reactivity to emotional faces. Although change in self-reported stress levels did not predict change in amygdala reactivity over time in the current study, the influence of general distress on amygdala reactivity is an important issue to be considered in future studies.

We did not find a main effect of group or time in DLPFC activity to emotional faces. Based on previous research (Maslowsky et al., 2010) we would expect to find an increase in PFC activation over time. Possibly, the effects were masked by the task design. It might be that PFC effects for time and group only appear when the cognitive load of the task corresponds better to depression/anxiety symptomatology, e.g. when using an emotion regulation task. Future research should further investigate these effects by using different task designs and a more representative cognitive load.

There are some limitations of the current study that should be taken into account. First, amygdala reactivity did not differ between groups before treatment, even though self-report showed that the clinical adolescents experienced severe problems related to depression and anxiety (Van Den Bulk et al., 2014). To examine the unique contribution of depression and anxiety on deviant patterns of brain activation we included a comorbid group of adolescents with a depressive or anxiety disorder. Studying adolescents with co-

morbid disorders matches the idea of the Research Domain Criteria project (RDoC) developed by NIMH (Insel et al., 2010). This approach aims at creating new guidelines for classifying psychopathology based on dimensions of, among others, neurobiological measures. However, the heterogeneity of our group may have confounded the result, although now the sample is a good representation of clinical practice in which comorbidity between depression and anxiety is high (Essau, 2008). Also, the sample size may have been too small to investigate the relation between changes in self-reported symptomatology and changes in amygdala activation over the course of treatment. Moreover, inclusion of state questions in the face-processing task may have attenuated amygdala involvement in the task, as it has been shown that more cognitively demanding tasks decrease emotional reactivity (Costafreda et al., 2008). Finally, the treatment protocol and duration varied between participants, causing session 3 to be at different time points in the individual course of treatment. Yet, we tested whether number of treatment sessions influenced the results and this was not the case. However, within the current study design we were not able to examine treatment effectiveness in relation to longitudinal changes in amygdala activation. Future research should extend our findings by including a larger sample of adolescents with depressive and anxiety disorders that are referred for several forms of treatment, e.g. structured CBT procedures and medication, and can be compared with a control group of adolescents. This will provide the opportunity to further investigate the influence of individual differences in depression and anxiety symptomatology and eliminates inter individual treatment effects.

To conclude, in contrast to adult studies (Clark, & Beck, 2010; Quide et al., 2012), but in line with previous research in adolescents (Maslowsky et al., 2010) our results indicated an increase in amygdala activation in adolescents with DSM-IV depressive and/or anxiety disorders over treatment, that paralleled a decrease in symptoms of depression and anxiety and a decrease in self-reported stress levels. These results provide new insights in possible time and treatment related changes in amygdala activation in

adolescents with a depressive and/or anxiety disorder and may suggest different treatment-related changes in amygdala reactivity in adolescents compared to adults. To our knowledge this is one of the first longitudinal fMRI studies including a relatively large sample of treatment naïve adolescents with a depressive/anxiety disorder. The results highlight the need for more longitudinal research investigating time related changes in brain activation specifically in adolescents. By acquiring more in-depth information about the neurobiological mechanisms of depression and anxiety we eventually may be able to increase treatment and intervention effectiveness.

Supplemental material

Behavioral analyses

Analyses of the reaction times and subjective ratings of the emotional faces task were examined over time and between groups using repeated measurement ANOVAs in SPSS 19 (see also supplemental figure 1). In case sphericity could not be assumed, a Greenhouse-Geisser correction (GG-corr.) was used. All post-hoc tests were Bonferroni corrected for multiple comparisons. Values deviating more than three standard deviations from the mean were considered outliers and removed from the analyses. Furthermore, expectation maximization was used when items in the RCADS (5 in total) and CDI (6 in total) were missing.

Behavioral data – reaction time and subjective scoring

The state question 'how afraid are you' resulted in a main effect of TP ($F_{(2,72)}$=5.23, p<.01) and a main effect of emotion ($F_{(2,72)}$=28.50, p<.001, GG-corr.). Subjective scorings were higher at TP1 (p<.05) and TP2(p<.05) than at TP3, participants reported being more afraid of fearful (p<.001) and neutral (p<.001) faces than for happy faces and reported more fear for fearful faces than for neutral faces (p<.005). The state question 'how happy are you?' resulted in a main effect of emotion ($F_{(2,80)}$=93.29, p<.001, GG-corr.) in which subjective scoring was higher for happy faces than for fearful and neutral faces (p's<.001). In addition, there was an emotion x group interaction ($F_{(2,80)}$=3.92, p<.05, GG-corr.): the clinical group reported being less happy when seeing fearful faces compared to the control group (p<.01). The state question 'How wide is the nose?' resulted in a main effect for TP ($F_{(2,80)}$=5.20, p<.01) and a main effect of emotion ($F_{(2,80)}$=418.60, p<.001). Subjective scorings for nose width were higher at TP1 compared to TP2 (p<.05), higher for happy faces than for fearful and neutral faces (p's<.001), and higher for fearful faces compared to neutral faces (p<.001).

The repeated measurement ANOVA for reaction time did not result in any main or interaction effects.

Whole brain activation patterns

At the whole brain group level, we performed a flexible (main effect of time and interaction effect session x group) and full (task related effects) factorial model. The analyses showed no significant effect of time related changes in activation patterns, no significant differences between groups and no significant interaction between group and session for any of the contrast (all faces>fixation, all fearful faces>fixation, all happy faces>fixation and all neutral faces>fixation). We also investigated task related effects in the complete sample of N=42 adolescents per session with the use of a full factorial model (all faces>fixation, all fearful faces>fixation, all happy faces>fixation and all neutral faces>fixation). The results of these contrasts show significant patterns of activation in brain area's previously related to emotional face processing (e.g. bilateral amygdala, bilateral insula and bilateral dorsolateral PFC; see also supplemental Table 1 and Figure 2).

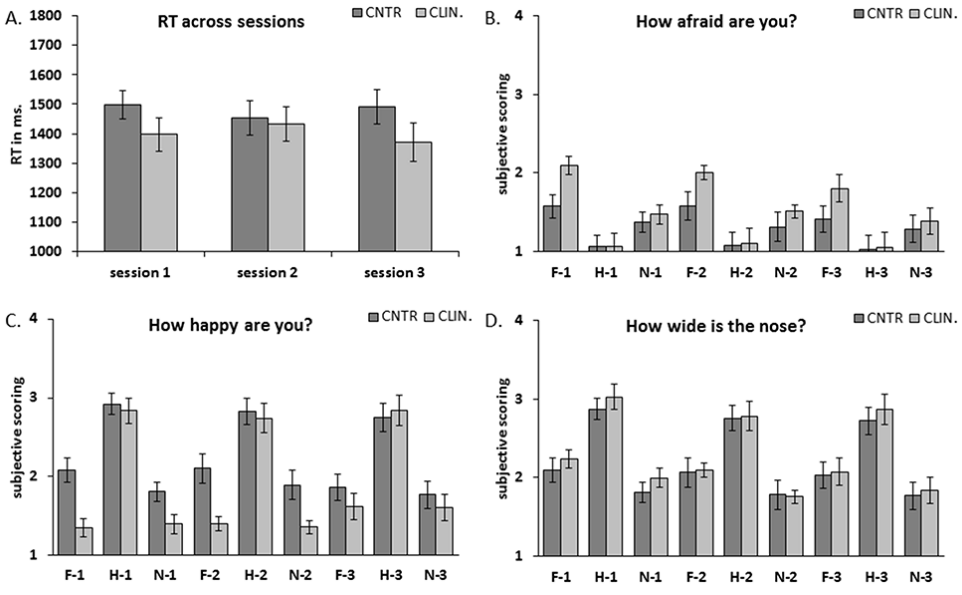

Figure S1. Behavioral scores for the emotional face-processing task. *(A) Mean reaction times in milliseconds collapsed across emotions and state questions. (B, C, D) represent mean subjective scoring per state question for both groups during all three sessions. CNTR=control group; CLIN.=clinical group; F=fearful faces; H=happy faces; N=neutral faces; 1=session 1; 2=session 2; 3=session 3.*

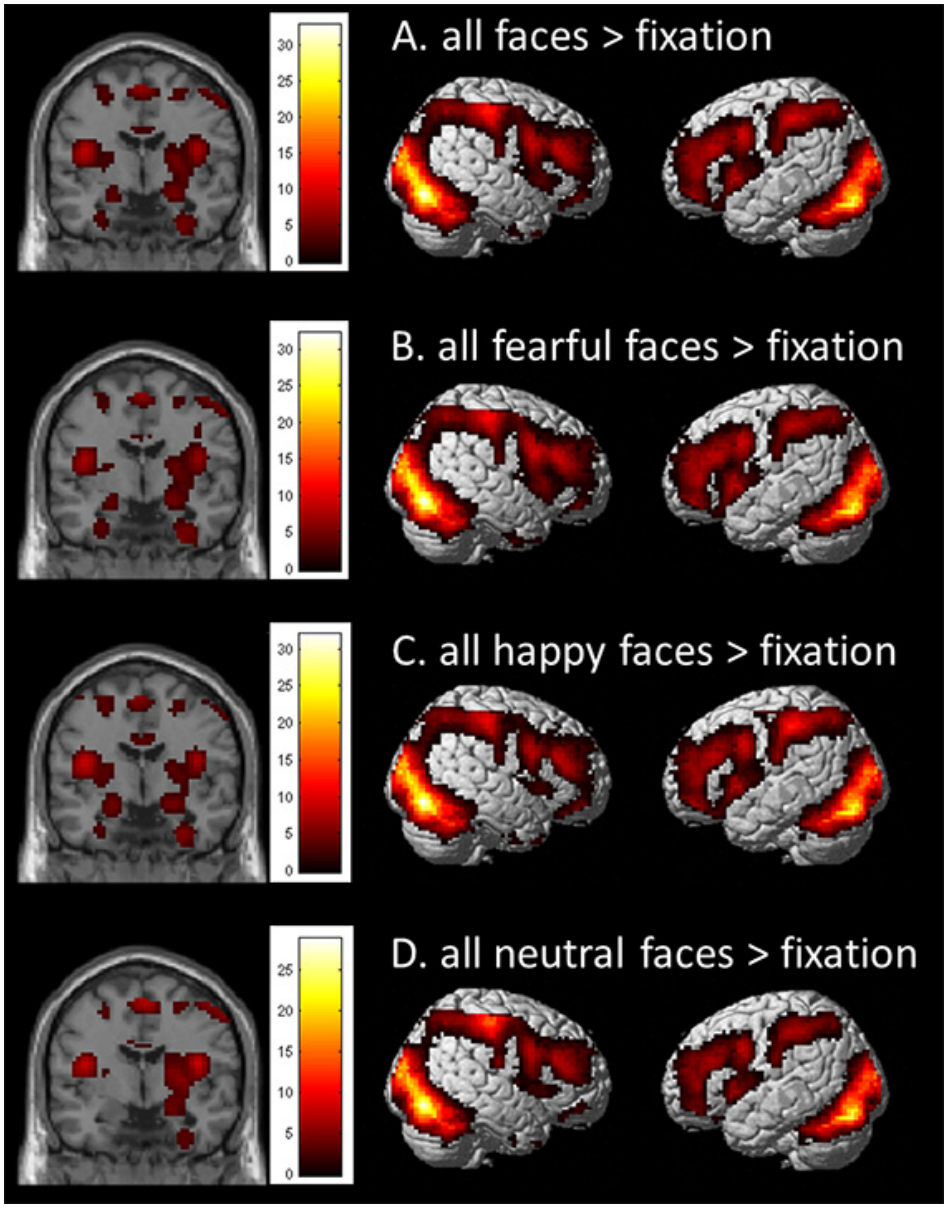

Figure S2. Whole brain activation patterns within the complete sample of N=42 adolescents per session for the contrasts: A. all faces > fixation, B. all fearful faces > fixation, C. all happy faces > fixation, and D. all neutral faces > fixation. *Coordinates represent significant peaks of activation at p<.05, FDR-corrected, 10 contiguous voxels and are listed in MNI space. *=p<.05 when corrected for multiple comparisons at cluster-level (FWE).*

Table S1. Whole brain activation patterns for the contrasts: A. all faces > fixation, B. fearful faces > fixation, C. happy faces > fixation and D. neutral faces > fixation. *Results are derived from a full factorial model. Regions represent significant peaks of activation at p<.05, FDR-corrected, 10 contiguous voxels and coordinates are listed in MNI space and represent peak values. *=p<.05 when corrected for multiple comparisons at cluster-level (FWE).*

Contrast	Region	Side	z-score	K_E	x	y	z	
A.								
All faces -fixation	Superior frontal gyrus	L	Inf	825	0	14	55	*
	Middle frontal gyrus	L	Inf	2939	-51	35	25	*
	Middle frontal gyrus	L	3.30	43	-27	-4	55	
	Cingulate gyrus	L	4.04	66	0	2	28	
	Postcentral gyrus	L	Inf	1289	-48	-34	49	*
	Postcentral gyrus	L	5.33		-57	-19	28	
	Precuneus	L	7.23		-27	-61	49	
	Middle occipital gyrus	R	Inf	12206	39	-76	-14	*
	Lingual gyrus	R	Inf		12	-82	-8	
	Lingual gyrus	L	Inf		-3	-82	-5	
	Insula	L	7.75		-39	14	4	
	Thalamus	L	7.76		-21	-31	-2	
	Uncus	R	6.18	109	33	-10	-35	
	Parahippocampal gyrus	L	4.81	98	-30	-10	-32	
	Parahippocampal gyrus	L	3.65		-21	-4	-17	
B.								
All fearful faces -fixation	Superior frontal gyrus	L	Inf	856	0	11	55	*
	Middle frontal gyrus	L	Inf	2962	-51	38	25	*
	Middle frontal gyrus	L	2.92	25	-27	-4	55	
	Inferior frontal gyrus	L	7.37		-48	47	7	
	Cingulate gyrus	L	3.52		0	2	28	
	Cingulate gyrus	L	2.43		-5	-10	31	
	Middle occipital gyrus	R	Inf	13673	39	-76	-14	*
	Lingual gyrus	R	Inf		12	-82	-8	
	Lingual gyrus	L	Inf		-3	-91	-5	
	Insula	L	7.57		-39	14	4	
	Thalamus	L	Inf	116	-21	-28	-2	
	Globus Pallidus	L	2.51		-15	-10	1	
	Putamen	R	5.81	178	33	-10	1	
	Uncus	L	4.60	125	-30	-10	-35	
	Uncus	L	2.88		-24	5	-32	
	Parahippocampal gyrus	L	3.73		-21	-4	-17	
	Cerebellar tonsil	L	4.01	10	-24	-40	-41	

C.								
All happy faces - fixation	Superior frontal gyrus	L	Inf.	792	0	14	52	*
	Middle frontal gyrus	L	Inf.		-51	35	25	
	Middle frontal gyrus	R	3.22	41	27	-4	55	
	Inferior frontal gyrus	L	7.84		-57	8	37	
	Cingulate gyrus	L	4.46	108	0	2	28	
	Middle temporal gyrus	R	2.88	47	36	14	-44	
	Middle occipital gyrus	R	Inf.	13182	39	-76	-14	*
	Lingual gyrus	R	Inf.		12	-82	-8	
	Lingual gyrus	L	Inf.		-3	-82	-5	
	Thalamus	L	Inf.	3173	-21	-31	-2	*
	Uncus	R	6.58	108	33	-10	-35	
	Cerebellar tonsil	L	3.72	10	-21	-40	-41	
D.								
All neutral faces - fixation	Superior frontal gyrus	L	Inf.	765	0	14	55	*
	Superior frontal gyrus	R	2.67		24	50	-17	
	Middle frontal gyrus	L	7.43	2096	-48	35	28	*
	Middle frontal gyrus	L	3.18	23	-24	50	-11	
	Middle frontal gyrus	L	3.09	18	-27	-4	52	
	Middle frontal gyrus	L	2.87	13	-24	29	-20	
	Middle frontal gyrus	R	2.85	94	42	50	-17	
	Middle frontal gyrus	R	2.62		30	35	-20	
	Inferior frontal gyrus	L	7.10		-63	11	31	
	Cingulate gyrus	L	3.17	31	-3	2	28	
	Postcentral gyrus	L	Inf.	1019	-48	-34	49	*
	Postcentral gyrus	L	5.25		-57	-19	28	
	Superior parietal lobule	L	6.08		-30	-58	52	
	Middle occipital gyrus	R	Inf.	11354	39	-76	-14	*
	Lingual gyrus	R	Inf.		6	-79	-5	
	Lingual gyrus	L	Inf.		-27	-79	-17	
	Insula	L	7.03		-39	-4	16	
	Thalamus	L	6.29	61	-21	-31	-2	
	Uncus	R	5.43	63	33	-10	-35	
	Parahippocampal gyrus	L	3.82	26	-30	-10	-32	