Bianca G. van den Bulk

The Affective Amygdala

towards a better understanding of adolescent depressive and anxiety disorders

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Chapter 1

General introduction

Introduction

Scope

The transition from childhood to adolescence is a vulnerable developmental period in which many external changes prevail: adolescents change schools, often start a part-time job and get new friends which become very important while they also become less dependent on their parents. Besides external changes, alterations at the biological level are remarkable as well, like the increase in pubertal hormones (Blakemore, Burnett, & Dahl, 2010) and the ongoing development of the brain (Giedd et al., 1999). For most adolescents, including myself, these changes just happen and have no further detrimental implications for their daily lives. Of course the majority of adolescents experience some struggles, for example having arguments with their parents about what time they need to be home or feeling extremely sad because their first relationship broke. For most adolescents, these feelings all pass by and do not cause ongoing problems. For some adolescents, however, this period is not without consequences and includes the onset of depressive and anxiety disorders. They struggle much more with their feelings and behaviors and experience problems in daily life. Although we know quite a lot about the development and persistence of depressive and anxiety disorders during adolescence and several randomized controlled trials indicated that the current treatment and intervention approaches are quite effective (Compton, Burns, Helen, & Robertson, 2002; Compton, March, Brent, Albano, Weersing, & Curry, 2004), there are many adolescents who do not benefit from treatment. Conducting research to examine the underlying neurobiological mechanisms of adolescent depression and anxiety can provide us with better insights about the exact mechanisms of these disorders and eventually might provide guidelines for the development of better treatment and intervention strategies (cognitive behavioral therapy or medication therapy).

Quite some studies have indicated that the amygdala is an important brain region related to emotion processing (Fusar-Poli et al., 2009; Wha-

len, Davis, Oler, Kim, Kim, & Neta, 2009) and it is shown that depressed and anxiety participants (both adults and adolescents) show increased amygdala activation when processing emotional faces when compared to healthy controls (Beesdo et al., 2009b; Monk et al., 2008a; Monk et al., 2008b; Perlman et al., 2012; Thomas et al., 2001a). The results of these studies suggest that the amygdala is a target region when performing research into the neurobiological mechanisms of depression and anxiety. Because depression and anxiety often have their onset during adolescence, it is important to further investigate differentiating patterns of amygdala activation within this specific period. Furthermore, efforts should be made to examine longitudinal changes in amygdala activation. Only these studies provide with important information about individual differences in the development of depressive and anxiety disorders. Therefore, we conducted a large longitudinal study examining cross-sectional and longitudinal differences in amygdala activation and connectivity in a sample of adolescents with depressive and anxiety disorders.

Depression and anxiety

Depression and anxiety are two of the most diagnosed psychiatric disorders during adolescence. Studies investigating prevalence rates indicated that approximately 10% of adolescents develop a depressive disorder and about 20-24% any anxiety disorder (Kessler et al., 2012a). The comorbidity between depressive and anxiety disorders is found to be high: for example a study by Essau (2008) investigate the comorbidity of depressive and anxiety disorders in adolescents and showed that about half of the adolescents with a depression diagnosis also fulfills the criteria for an anxiety disorder. This high comorbidity and the large overlap in emotion related symptomatology increases the need to examine both disorder groups together.

The presence of a depressive or anxiety disorder all too often causes substantial problems in the lives of adolescents. Research indicated that depression and anxiety lead to a decrease in the rating of quality of life and af-

fects the thoughts, feelings and behaviors of these adolescents, which influences their daily life (Zisook et al., 2007). When taking comorbidity between depression and anxiety into account the perspectives are even worse: Lewinsohn and colleagues (Lewinsohn, Gotlib, & Seeley, 1995) reported poorer global functioning, more academic problems and a higher risk for attempted suicide in a comorbid depressed-anxious group compared to a 'pure' depression group. Depression and anxiety not only cause problems during adolescence, but also continues to affect people into adulthood: Adolescents with a depressive or anxiety disorder have a 2- to 3-fold increased risk for having a depressive or anxiety disorder during adulthood (Pine, Cohen, Gurley, Brook, & Ma, 1998). This suggests that these disorders are relatively complicated and are hard to treat, which makes it even more important to conduct studies that include both depressed and anxious adolescents.

One often used form of treatment for depression and anxiety in adolescents is cognitive behavioral therapy (CBT). Individuals with a depression or anxiety disorder often have maladaptive thoughts, feelings and behaviors. By changing and exploring individuals can learn how to adapt their thoughts, feelings and behaviors. It challenges an individual to replace the maladaptive thoughts, feelings and behaviors with adaptive ones. Techniques that are applied within CBT include exposure and cognitive restructuring (Compton et al., 2004). Research has shown that CBT is a quite effective form of treatment for both depression and anxiety, with comparable levels of effectiveness as treatment with medication (Compton et al., 2002; Compton et al., 2004). However, not everyone benefits from CBT and more research is necessary to further investigate why some individuals do benefit while others do not. One way of doing that is by examining the neurobiological mechanisms of depression and anxiety with the use of functional Magnetic Resonance Imaging (fMRI).

Depression and anxiety in the brain

When conducting research on the neurobiological mechanisms of de-

pressed and anxious adolescents, it is important to take brain development into account. Research indicated that there are ongoing changes in gray matter and white matter that continue into adulthood (Giedd et al., 1999) and are related to enhanced plasticity in cognitive and emotional functioning (Steinberg, 2005). Furthermore, it is known that several brain regions or networks follow distinct maturational trajectories: the cognitive control regions in the prefrontal cortex develop at a slower pace than regions related to the processing of affective information in the limbic system (Gogtay et al., 2004). Because of these distinct maturational trajectories, an imbalance arises in which the limbic 'emotional' regions are further developed than the prefrontal 'control' regions (Somerville, & Casey, 2010). It appears that this imbalance is largest during adolescence and possibly plays a role in the onset of depression and anxiety during adolescence (Casey, Jones, & Hare, 2008; Somerville, & Casey, 2010).

One brain region within the limbic system that is important for the processing of emotional stimuli is the amygdala. The amygdalae are two almond shaped groups of nuclei located deep in the brain. It is known that the amygdala is involved in the processing of emotional stimuli, more specifically emotional faces (Costafreda, Khanna, Mourao-Miranda, & Fu, 2009; Fusar-Poli et al., 2009; Whalen et al., 2009). Furthermore, the amygdala is part of the social information-processing network and the overlapping face processing network (Scherf, Behrmann, & Dahl, 2012). Finally, research indicated that the amygdala is important for learning associations between a stimulus and its emotional significance, for example learning the association between seeing a spider and being afraid and careful (Tottenham, Hare, & Casey, 2009a). Early on, it was thought that the amygdala only was involved in the processing of negative stimuli. However, nowadays the amygdala is known to be involved in the processing of both negative and positive stimuli (Davis, & Whalen, 2001; Somerville, Kim, Johnstone, Alexander, & Whalen, 2004; Van Den Bulk et al., 2013). Meta-analyses by Costafreda and colleagues (2008) and Fusar-Poli and colleagues (2009) showed that the amygdala is

most strongly activated in response to fearful and disgusted faces, but also in response to happy and neutral faces. Studies investigating developmental differences in amygdala activation in response to emotional faces indicated that there is an increase in amygdala reactivity during adolescence (Baird et al., 1999; Guyer et al., 2008; Pfeifer et al., 2011). For example, a study by Hare (Hare, Tottenham, Galvan, Voss, Glover, & Casey, 2008) indicated that adolescents showed higher levels of amygdala activation compared to children and adults when performing an emotional go no-go task. These findings seem to correspond to the theory of an imbalance in the development of prefrontal cortex regions and limbic regions (Somerville, & Casey, 2010).

Overall, the amygdala is important for the processing of emotional stimuli. Because it is thought that in depression and anxiety the primary emotional response is exaggerated and not effectively controlled by prefrontal cortex regions (Mayberg, 1997), the amygdala might be important target regions when examining the neurobiological mechanisms of depression and anxiety. There are some studies that investigated amygdala reactivity to emotional stimuli in adolescents with depressive and anxiety disorders (Hulvershorn, Cullen, & Anand, 2011; Monk, 2008). In general these studies reported heightened patterns of amygdala response to emotional faces. The results of studies including depressed adolescents are somewhat more inconsistent than those including anxious adolescents. For example, a study by Roberson-Nay and colleagues (2006) found an increase in amygdala response for depressed adolescents compared to healthy controls while another study including depressed adolescents reported a decrease in amygdala response (Thomas et al., 2001a). Studies including adolescents with an anxiety disorder show much more consistency: when using an emotional face processing task several studies reported an increase in amygdala activation in response to emotional faces for the anxious adolescents when compared with healthy controls (Mcclure et al., 2007b; Monk et al., 2008b). Even though the results of studies including depressed adolescents are somewhat inconsistent, in general increased patterns of amygdala activation in response to

emotional stimuli are found for depressed and anxious adolescents. These findings suggest that increased amygdala activation might be an underlying neurobiological mechanism of depression and anxiety. This suggestion is supported by some research that investigated the relation between differentiating patterns of amygdala activation and levels of self-reported anxiety symptoms, symptom severity and diagnoses (Ball et al., 2012; Monk et al., 2003a; Stein, Simmons, Feinstein, & Paulus, 2007; Thomas et al., 2001a). These studies indicated that there is a positive relation between levels of self-reported anxiety symptoms and amygdala activation.

Although some research has indicated that adolescents with depressive and anxiety disorders show heightened patterns of amygdala activation, more research is necessary to further investigate the neurobiological mechanisms of depression and anxiety. Up till now, most research has been performed in adults and the amount of research performed in adolescents is still relatively low. Furthermore, not much is known about longitudinal changes in amygdala activation in depressed and anxious adolescents: most studies used data of just one fMRI session and compared a depressed/anxious group with a healthy control group (Canli et al., 2005; Mcclure et al., 2007a). Performing longitudinal research provides us with the opportunity to examine individual differences and changes in amygdala activity and depression/anxiety symptomatology and thereby provides us with better insights in individual trajectories and the influence and effectiveness of treatment.

Objectives and approach

Goal

Because of the large increase in depression and anxiety diagnoses during adolescence and the persistence of these disorders into adulthood it is worthwhile to further investigate the underlying neurobiological mechanisms of adolescent depression and anxiety. Possibly, these studies will provide further insight in the mechanisms of these disorders and directions for future studies that might lead to better treatment and intervention strategies.

In this thesis we investigate the neurobiological mechanisms of depression and anxiety with a focus on amygdala functioning. The main objectives for this thesis were three fold. First, to further examine whether adolescents with depressive and anxiety disorders show increased patterns of amygdala activation compared to healthy controls when performing an emotional face-processing task. In addition, we also investigated whether depressed and anxious adolescents show less habituation of amygdala activation in response to emotion faces. Second, to examine the test-retest reliability of the fMRI signal in brain regions related to face processing (bilateral amygdala, bilateral lateral prefrontal cortex and visual cortex). Third, to study longitudinal changes in amygdala activity (task based activation) and connectivity (based on resting state analyses) in a sample of depressed and anxious adolescents who were referred for cognitive behavioral therapy based treatment. Within these studies we also explored the relation between changes in brain activity/connectivity and change in self-reported symptomatology.

Approach – the EPISCA study

All studies described in this thesis were part of the larger EPISCA study. EPISCA stands for 'Emotional Pathways' Imaging Study in Clinical Adolescents' and is a large longitudinal study in which three clinical settings collaborated: Curium-LUMC, 'GZZ kinderen en jeugd Rivierduinen' and 'het kinder en jeugd trauma centrum Haarlem'. The study included a group of treatment naïve adolescents with a DSM-IV depression or anxiety disorder, a group of adolescents who experienced childhood sexual abuse and who were seeking help for trauma related symptomatology and a group of normally developing adolescents without psychopathology. They all were between 12 and 19 years old.

The overall goal of EPISCA was to examine differences between these groups in the neurobiological mechanisms related to emotion processing and emotion regulation. To do this, all adolescents were scanned three times

in a six-month period. In between scan sessions the adolescents from the two clinical groups received treatment as usual which was based on cognitive behavioral therapy or EMDR (eye movement desensitization reprocessing). Adolescents from the control group were scanned within the same time interval but did not receive treatment. During a scan session several MRI parameters were collected: task based fMRI (emotional face processing task), resting state fMRI, high-resolution structural scan and Diffusion Tensor Imaging. Besides the scan sessions, we also administered several interviews (session 1 and 3), questionnaires (each session; both for the adolescents and their parents) and subtests of an intelligence test (session 1).

Outline of the chapters

The first part of this thesis describes two task-based fMRI studies examining group differences in amygdala activation during an emotional face-processing task. In ***chapter 2***, we included a group of adolescents with depressive and anxiety disorders and a healthy control group. All adolescents were scanned with fMRI and while in the scanner they performed an emotional face-processing task. This study aimed to investigate the underlying neurobiological mechanisms of depression and anxiety in relation to emotional face processing. By using an emotional face-processing task we were able to investigate differentiating patterns of amygdala activation and its relation to individual differences in self-reported depression and anxiety symptoms.

Chapter 3 concerns a study including three groups of participants: a group of depressed and anxious adolescents, a group of adolescents who experienced childhood sexual abuse and a healthy control group. In this study we focused on group differences in the habituation of amygdala activation in response to emotional faces. We included two clinical groups to be able to investigate whether these groups showed different patterns of amygdala habituation in response to emotional faces. Depression/anxiety and childhood sexual abuse (CSA) share a lot of clinical features mainly related to mood and anxiety symptomatology. However, CSA also has a unique component namely the experience of a traumatic event.

Chapters 4 and 5 describe longitudinal fMRI studies using an emotional face-processing task. Performing longitudinal fMRI studies provides us with the opportunity to examine individual changes in brain and behavioral functioning, which is important to understand the influence of development and treatment on brain functioning and the development and persistence of psychiatric disorders. We first examined the test-retest reliability of brain activation in a sample of healthy adolescents ***(chapter 4)***. These adolescents were scanned three times in a six-month period and during each scan session they performed an emotional face-processing task. More specifically, we focused on the within-subject reliability of amygdala, lateral prefrontal

cortex and visual cortex activation during an emotional face-processing task. These analyses provide us information about whether activation in a specific region at time point 1 is comparable to activation in that same region at time point 2 and 3 within individuals.

Next, we performed a longitudinal study investigating changes in amygdala and prefrontal cortex activation in depressed and anxious adolescents and a healthy control group, which is described in ***chapter 5***. In this study we examined whether amygdala and prefrontal cortex activation changed over a six-month period during which the depressed/anxious adolescents received treatment as usual. Furthermore, we were interested in the relation between changes in brain activation and changes in self-reported symptomatology. We reasoned that brain activation might change under the influence of treatment: when treated adolescents report fewer symptoms which might be visible in the brain by means of a change in activation in important brain regions related to depression and anxiety like the amygdala and prefrontal cortex.

In ***chapter 6*** we shifted focus to resting state fMRI. The main objective of the study presented in ***chapter 6*** was to examine longitudinal changes in resting state functional connectivity in a sample of depressed and anxiety adolescents who were referred for treatment and a sample of healthy controls. We used a seed-based approach and focused on connectivity between the bilateral amygdala and the rest of the brain. Performing these analyses gives us more information about the connectivity between the amygdala and other brain regions and whether these changes are related to changes in self-reported symptomatology.

The last chapter ***(chapter 7)*** does not describe an empirical study but summarizes the findings of ***chapters 2-6*** (concluding remarks), provides some general considerations in relation to the studies described in the thesis and discuss the results in relation to the main goals stated in the introduction.

The following papers have resulted from this thesis

van den Bulk, B.G., Meens, P.H.F., van Lang, N.D.J., de Voogd, E.L., van der Wee, N.J.A., Rombouts, S.A.R.B., Crone, E.A., Vermeiren, R.R.J.M. *Amygdala activation during emotional faces processing in adolescents with affective disorders: the role of underlying depression and anxiety symptoms.* Frontiers in Human Neuroscience, 8, #393. ***(chapter 2)***

van den Bulk, B.G., Somerville, L.H., van Hoof, M.J., van Lang, N.D.J., van der Wee, N.J.A., Crone, E.A., Vermeiren, R.R.J.M. *Habituation effects during emotional face processing in adolescents with internalizing disorders, sexually abused adolescents and healthy controls.* Submitted. ***(chapter 3)***

van den Bulk, B.G., Koolschijn, P.C.M.P, Meens, P.H.F., Lang, N.D.J., van der Wee, N.J.A., Rombouts, S.A.R.B., Vermeiren, R.R.J.M. & Crone, E.A. (2013). *How stable is activation in the amygdala and prefrontal cortex in adolescence? A study of emotional face processing across three measurements.* Developmental Cognitive Neuroscience, 4, 65-74. ***(chapter 4)***

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.* Submitted. ***(chapter 5)***

van den Bulk, B.G., van der Werff, S.J.A., Aghajani, M., Granettia, M., van Lang, N.D.J., van der Wee, N.J.A., Rombouts, S.A.R.B., Crone, E.A., Vermeiren, R.R.J.M. *Longitudinal changes in resting-state functional connectivity in depressed and anxious adolescents in relation to treatment.* Submitted. ***(chapter 6)***

Chapter 2

Amygdala reactivity in response to emotional faces in depressed and anxious adolescents

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Amygdala activation during emotional faces processing in adolescents with affective disorders: the role of underlying depression and anxiety symptoms.
Frontiers in Human Neuroscience, 8, #393.

Abstract

Depressive and anxiety disorders are often first diagnosed during adolescence and it is known that they persist into adulthood. Previous studies often tried to dissociate depressive and anxiety disorders, but high comorbidity makes this difficult and maybe even impossible. The goal of this study was to use neuroimaging to test what the unique contribution is of depression and anxiety symptomatology on emotional processing and amygdala activation, and to compare the results with a healthy control group. We included 25 adolescents with depressive and/or anxiety disorders and 26 healthy adolescents. Participants performed an emotional face processing task while in the MRI scanner. We were particularly interested in the relation between depression/anxiety symptomatology and patterns of amygdala activation. There were no significant differences in activation patterns between the control group and the clinical group on whole brain level and ROI level. However, we found that dimensional scores on an anxiety but not a depression subscale significantly predicted brain activation in the right amygdala when processing fearful, happy and neutral faces. These results suggest that anxiety symptoms are a better predictor for differentiating activation patterns in the amygdala than depression symptoms. Although the current study includes a relatively large sample of treatment naïve adolescents with depression/anxiety disorders, results might be influenced by differences between studies in recruitment strategies or methodology. Future research should include larger samples with a more equal distribution of adolescents with a clinical diagnosis of depression and/or anxiety. To conclude, this study shows that abnormal amygdala responses to emotional faces in depression and anxiety seems to be more dependent on anxiety symptoms than on depression symptoms, and thereby highlights the need for more research to better characterize clinical groups in future studies.

Introduction

Depressive and anxiety disorders often have their onset during adolescence; adolescent prevalence rates for mood disorders are estimated to be around 10%, and rates for any anxiety disorders are as high as 24.9% (Kessler et al., 2012a) and Costello and colleagues (2011) found a continued increase in the prevalence of depressive and anxiety disorders from adolescence to adulthood. It is also known that the comorbidity between depressive and anxiety disorders during adolescence is high. For example, within a clinical group of adolescents with a depression diagnosis, almost half also had an anxiety disorder (Essau, 2008). Comorbidity carries clinical relevance because it predicts a more negative outcome: Lewinsohn and colleagues (1995) reported a poorer global functioning in a comorbid depressed-anxious group than in the 'pure' depression group.

Elucidating the underlying neurobiological mechanisms of these disorders may be crucial to fully understand the negative outcomes in adolescent depression and anxiety. FMRI studies in particular can be helpful for relating disturbed psychological and neurobiological processes with clinical severity. A better relationship between neuroscience and clinical research may result in better diagnoses and increased understanding in the future (Pine, Guyer, & Leibenluft, 2008). However, studies investigating underlying neurobiological mechanisms generally focus on adolescents with a specific disorder, without fully taking comorbidity and dimensionality into account (e.g. (Brotman et al., 2007; Mcclure et al., 2007b; Monk et al., 2008b; Perlman et al., 2012; Strawn et al., 2012a; Yang et al., 2010). In the current study we included a group of adolescents with depression and/or anxiety disorders and investigated the neurobiological correlates of emotional face processing with special regard for the individual differences and symptom dimensionality of these disorders.

Adolescents with depressive or anxiety disorders are known to show similar disturbed emotion perception (Thomas et al., 2001a) and regulation (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & Van Ijzendoorn,

2007; Shechner et al., 2012). In the last decade, a number of task-based fMRI studies have focused on the brain mechanisms related to emotion processing in depressed and anxious adolescents (e.g. (Mcclure et al., 2007b; Monk et al., 2008a; Monk et al., 2008b; Perlman et al., 2012; Thomas et al., 2001a). In these studies, participants are often asked to passively view, label or rate emotional faces. For instance, in a study by Mingtian and colleagues (2012) adolescents were asked to recognize and match faces by emotional expression, while in other studies, they were asked to interpret emotional faces by focusing their attention to their own internal state or to other more external objectives in the face (i.e., Mcclure et al., 2007b). In these studies, the amygdala is consistently reported to play an important role in the processing of emotional faces (Fusar-Poli et al., 2009; Whalen et al., 2009). The amygdala is part of the social information processing network and the overlapping face processing network (Scherf et al., 2012). It is known that the amygdala plays an important role in learning associations between a stimulus and its emotional significance (Tottenham et al., 2009a). Prior research indicated that amygdala activity increases in response to both positive and negative face stimuli (Davis, & Whalen, 2001; Somerville et al., 2004; Van Den Bulk et al., 2013). Meta-analyses show that the amygdala is most strongly activated for fearful and disgusted faces and to a somewhat lesser extent for happy and neutral faces (Costafreda et al., 2008; Fusar-Poli et al., 2009). However, amygdala activation not only depends on emotional valence but also on the cognitive demands of a paradigm. For example, explicit face processing (e.g. directing attention to emotional features of the face) increases bilateral amygdala activation relative to implicit face processing (e.g. diverting attention to nose width; (Fusar-Poli et al., 2009). Also, amygdala activation decreases when participants are instructed to label faces or to indicate their own subjective feeling compared to a passive viewing condition (Costafreda et al., 2008). Overall, the amygdala is strongly involved in emotion processing and an important brain area for underlying neural correlates of depressive and anxiety disorders.

Studies investigating the neurobiological correlates of emotional face processing in adolescents with depressive disorders have found inconsistent associations with amygdala activation (Hulvershorn et al., 2011; Monk, 2008). For example, a study by Thomas and colleagues (2001a) showed blunted amygdala response to fearful faces in a group of adolescent girls with a major depressive disorder, while two other studies showed heightened amygdala response in mixed gender groups with a major depressive disorder (Roberson-Nay et al., 2006) or youths at high risk for depression (Monk et al., 2008a). In contrast, studies in anxious adolescents were much more consistent, as multiple studies reported heightened amygdala responses to fearful and angry faces (Mcclure et al., 2007b; Thomas et al., 2001a). For example, a study by Monk and colleagues (Monk et al., 2008b), in which they scanned youths with generalized anxiety disorder, showed heightened patterns of amygdala activation in response to briefly presented masked angry faces. Based on these studies, differentiating patterns of amygdala activation during face processing tasks seems to be related to depressive and anxiety disorders and it might indicate an underlying neurobiological mechanism of depression and anxiety. However, it is not yet completely clear what the unique contribution of depression or anxiety is too these differentiating activation patterns.

Only a few clinical studies in adolescents investigated the relation between amygdala activation and symptom severity (Thomas et al., 2001a) or the difference in patterns of amygdala activation between depressed and anxious adolescents (e.g. Beesdo et al., 2009b) when studying emotional face processing. For example, Thomas and colleagues (2001a) correlated daily self-reported anxiety with amygdala activation in adolescents with depressive or anxiety disorders. They found a significant positive correlation between daily reported anxiety and activation in the amygdala. More recently, Beesdo and colleagues (2009b) reported common patterns of amygdala activation between adolescents with depression and adolescents with anxiety during active fearful face processing (focused attention on internally experienced

fear). However, during passive viewing the anxious adolescents showed hyperactivation of the amygdala while depressed adolescents showed hypoactivation. These results indicate that there are common and distinct neural patterns of amygdala activation between depression and anxiety that might be explained by task design or disorder-specifics characteristics.

There are also a few studies that investigated the relation between self-reported levels of anxiety (within the normal range) and amygdala activation in non-clinical adolescent or (young) adult samples (Ball et al., 2012; Monk et al., 2003a; Somerville et al., 2004; Stein et al., 2007). In general the results of these studies showed a positive relation between levels of anxiety and amygdala activation suggesting that levels of anxiety influence amygdala activation.

Although there are some studies that investigated the relation between depression, anxiety and amygdala activation in both clinical and non-clinical samples, more research is necessary to further delineate the unique contributions of depression and anxiety symptomatology to differentiating patterns of amygdala activation during face processing. This will aid in understanding individual differences between adolescents who have (comorbid) depressive or anxiety disorders and how they perceive and regulate negative, neutral and positive emotions. Also, using a dimensional approach is in line with the Research Domain Criteria approach (Insel et al., 2010), which is intended to provide a new classification framework for research into psychopathology. Therefore, the purpose of the present study is to investigate the underlying neurobiological correlates of emotional face processing in treatment-naïve adolescents with a depressive and/or anxiety disorders and in matched healthy controls. We were specifically interested in whether there is a relation between severity of depression or anxiety symptoms and activation patterns in the amygdala within a comorbid depression/anxiety group. This creates the opportunity to investigate depression and anxiety dimensionally instead of only using a categorical distinction between the two disorder groups. Based on previous studies we expected heightened patterns

of amygdala activation in the clinical group compared to the control group. Based on prior findings by Thomas et al. (Thomas et al., 2001a), we also expected a strong positive relation between self-reported anxiety symptoms and amygdala activation (Ball et al., 2012; Monk et al., 2003a; Somerville et al., 2004; Stein et al., 2007).

Methods

Participants

Functional MRI data were collected for 25 treatment naïve adolescents with a clinical diagnoses of a current DSM-IV depressive or anxiety disorder (Mean $Age_{(SD)}=15.44_{(1.53)}$, 21 females) and 26 healthy controls (Mean $Age_{(SD)}=14.65_{(1.55)}$, 23 females). The sex distribution was unequal (see Table 1) with a higher number of females than males due to the focus on internalizing disorders, which occur more often in females than in males. Within the clinical group 17 adolescents were diagnosed with a depressive disorder, 6 with an anxiety disorder and 2 with an adjustment disorder with depression and anxiety characteristics. All adolescents took part in the larger EPISCA study (Emotional Pathways' Imaging Study in Clinical Adolescents).

The adolescents from the clinical group were recruited in outpatient departments of two child and adolescent psychiatric institutes. The inclusion criteria were: having a clinical diagnosis of any depression or anxiety disorder, being referred for regular CBT-like psychotherapy, and being treatment naïve. They were excluded when other primary diagnoses were present or when they used psychotropic medications. The healthy control group adolescents 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.

Table 1. Group characteristics for the clinical and control group.

	Clinical group (N = 25)		Control group (N = 26)		
	N		**N**		**p**
Females	21 / 4		23 / 3		n.s.
	Mean	**SD**	**Mean**	**SD**	**p**
Age	15.44	1.53	14.65	1.55	n.s.
Full scale IQ	105	8.73	106	7.77	n.s.
Clinical DSM-IV diagnoses:	**N**	**%**			
Depression	7	13.7			
Dysthymia	10	19.8			
GAD	3	5.9			
SAD	2	3.9			
Adjustment disorder with dep./anx.	2	3.9			
Anxiety disorder NOS	1	2			
CDI†:	**Mean**	**SD**	**Mean**	**SD**	**p**
Total score	18.86	9.24	4.56	3.40	p < .001
RCADS	**Mean**	**SD**	**Mean**	**SD**	**p**
Total of five anxiety scale scores	31.65	14.46	14.85	12.83	p < .001

† CDI questionnaire data was missing for one participant with an adjustment disorder with depression characteristics, resulting in N=24 for the total sample. * RCADS anxiety subscale questionnaire data was missing for three participants (one with an adjustment disorder with depression and anxiety characteristics and two with a depressive disorder) resulting in N=22 for the total sample.

All participants met the following inclusion criteria: aged between 12 and 19, estimated full scale IQ≥80, right-handed, normal or corrected-to-normal vision, sufficient understanding of the Dutch language, no history of neurological impairments and no contraindications for MRI testing.

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). Both the clinical group ($Mean_{(SD)}=105_{(8.73)}$) and the control group ($Mean_{(SD)}=106_{(7.63)}$) scored in the average range. Overall, there were no significant differences between the

groups considering age ($F_{(1,49)}$=2.73, p=.105), estimated full scale IQ ($F_{(1,49)}$=.368, p=.547) and sex ($\chi 2_{(1)}$=.214, p=.642), and all participants were drug- and treatment naive.

Ten additional participants (clinical N=5, control N=5) were excluded from the analyses due to: unforeseen clinical features (N=1 control), use of medication (SSRI's; N=1 clinical), technical problems during scanning (N=3 clinical, N=1 control), excessive head movement (>3 mm, N=2 control) or anomalous findings reported by the radiologist (N=1 clinical, N=1 control).

Informed consent was obtained by participants, and by parents and participants in case of minors. The adolescents received a financial compensation including travel expenses for 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

Participants of the clinical group were included if they were diagnosed with any current DSM-IV depressive or anxiety disorder following clinical assessment by a child- and adolescent psychiatrist. Categorical DSM-IV diagnoses were further assessed with the Anxiety Disorders Interview Schedule (ADIS) for children and parents (Silverman, & Albano, 1996). In addition, 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, Yim, Moffitt, Umemoto, & Francis, 2000). The CDI is a self-report questionnaire with 27 items that correspond with dimensions of DSM-IV depressive disorders, and is scored on a 3-point Likert scale (0=*absence of symptomatology* to 2=*severe symptomatology*). The RCADS is a self-report questionnaire with 47 items that correspond with dimensions of DSM-IV depressive and anxiety disorders. The items are descriptive statements that are scored on a 4-point Likert scale (0=*never* to 3=*always*). In the current study, we only used the total score of the five RCADS anxiety sca-

les. For the control group, the same clinical instruments were used. Control group adolescents were excluded when they fulfilled the criteria for a DSM-IV diagnosis (ADIS interview) or had sub-clinical scores on clinical questionnaires.

Both questionnaires showed high levels of internal consistency: alpha for the CDI total scale .94 and for the RCADS anxiety subscale was .95.

Task

We administered an emotional faces task that was originally developed by McClure and colleagues (Mcclure et al., 2007b; 2003a) and that showed robust differences in brain activation patterns between participants with and without internalizing disorders. We described the adaptations we made in detail previously (Van Den Bulk et al., 2013). In short, the task consisted of three constrained (questions: 'how afraid are you?', 'how happy are you?' and 'how wide is the nose?') and one unconstrained (passive viewing) attention condition. After condition presentation, participants viewed 21 emotional faces (fearful, neutral or happy facial expression with an equal distribution of male and female actors) per attention condition, which they had to rate on a four-point rating scale (1. not at all, 2. a little, 3. quite and 4. very). During the task, reaction times and subjective scorings 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 attention conditions for 4000 milliseconds which was followed by a centrally located fixation cross with a jittered interval between 500 and 6000 milliseconds. Thereafter, one of the pictures was shown for 3000 milliseconds again followed by a centrally located fixation cross (Figure 1). Nothing happened when participants did not respond within 3000 milliseconds and those trials were recorded as missing trials (1.53% in total), which were not included in the analyses. We are aware of the ongoing debate whether the term "neutral" faces exist, or whether the term "ambiguous" faces should be used (Tahmasebi et al., 2012), but for consistency with our previous paper we use the term 'neutral' faces.

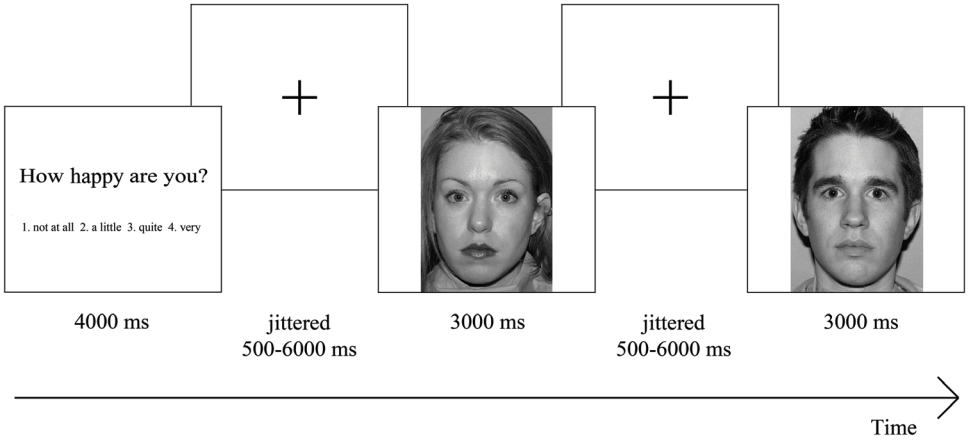

Figure 1. Overview of task design. *Participants were presented with an attention condition, followed by a centrally located fixation cross. Thereafter, they saw one of the emotional faces, again followed by a centrally located fixation cross, after which another emotional face was shown. Participants had to rate each emotional face on a four-point rating scale ranging from 'not at all' to 'very', based on the presented attention condition.*

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 participants by means of a mirror mounted to the head coil assembly. First, a localizer was obtained for each participant. Subsequently, T2*-weighted Echo-Planar Images (EPI) (TR=2.2s. TE=30ms, 80x80 matrix, FOV=220, 38 slices of thickness 2.72 mm) were obtained during three functional runs of 192 volumes each. Each run had two additional scans at the start that 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: repetition time 9.8 ms; echo time 4.6 ms; flip angle 8°; 140 sagittal slices; no slice gap; FOV= 224; 1.17 x 1.17 x 1.20 mm voxels; duration 4:56 minutes.

Behavioral analyses

The effects of emotional faces on subjective scoring were examined for each attention condition separately, using group (2 levels) x emotion (3 levels) repeated measurement ANOVAs in SPSS 19. The scores were analyzed separately for each attention condition, because values of the scores represent different interpretations for each condition. For reaction time, one repeated measurement ANOVA was performed with a group (2 levels) by emotion (3 levels) design. In case sphericity was not assumed, Greenhouse-Geisser correction (GG-corr.) was applied. No outliers were detected in the task data and the questionnaire data.

fMRI analyses

We used SPM8 (Welcome Department of Cognitive Neurology, London) to analyze the acquired data. Data was preprocessed using the following steps: 1. realignment of functional time series to compensate for small head movements and differences in slice timing acquisition, 2. registration and normalization of functional volumes (from EPI to individual structural T1 and thereafter to the T1 template), 3. spatial smoothing of 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, Kollokian, Kwan, & Evans, 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 were analyzed using the general linear model in SPM8. The fMRI time series were modeled by a series of events convolved with a canonical hemodynamic response function (HRF). The attention conditions were modeled separately as 4000 millisecond events and were added as covariates of no interest. The picture presentation of each emotional face

was modeled as a zero duration event. In the model, the picture presentation was further divided in twelve separate function trials (four attention conditions by three expressed emotions). 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 (e.g. all faces vs. fixation and fearful faces vs. fixation). The resulting contrast images, computed on a subject-by-subject basis, were submitted to group analyses. At the group level, we performed a full factorial model in which we included a factor called condition (12 levels, condition > null contrasts) and a factor called group (2 levels). We were mainly interested in the main effect of group, the interaction effect of group x condition and the overall task effects. Task-related responses were considered significant if they consisted of at least 10 contiguous voxels at a corrected threshold of $p<.05$ (FDR corrected).

We used the MarsBaR toolbox for use with SPM8 (http://marsbar.sourceforge.net/; Brett, Johnsrude, & Owen, 2002) to perform region of interest (ROI) analyses to further investigate patterns of activation. ROIs were defined based on a priori hypothesis about the bilateral amygdala (anatomically defined). No outliers were detected in the ROI output.

Correlation and regression analyses

To examine the relation between symptom severity and amygdala activation patterns, we correlated scores of the anxiety scale of the RCADS and the total CDI score with the ROI percent signal change values of the whole anatomically defined amygdala in SPSS. Furthermore, we performed stepwise regression analyses with percent signal change values as a dependent variable and the demeaned scores of the RCADS anxiety scale, CDI total scale and an interaction term of these two as independent variables. The correlation and regression analyses were performed for each emotion separately, collapsed across attention conditions, resulting in three regression analyses.

There were no outliers in the data (i.e., deviating >3 standard deviations) and expectation maximization was used when items in the RCADS (3 in total) and CDI (6 in total) were missing.

Results

Behavioral data

Subjective rating

The repeated measurement ANOVA for the condition 'how afraid are you?' resulted in a main effect of emotion ($F_{(2,98)}$=36.66, p<.001, GG-corr.), with higher subjective scorings for fearful faces than for neutral and happy faces (resp. p<.005 and p<.001) and higher scorings for neutral faces compared to happy faces (p<.001). Furthermore, there was a trend for an interaction effect between emotion and group ($F_{(2,98)}$=3.33, p=.054, GG-corr.), with higher scorings for fearful faces in the clinical group compared to the control group. The ANOVA for the condition 'how happy are you?' resulted in a main effect of emotion ($F_{(2,98)}$=100.53, p<.001, GG-corr.) and a main effect for group ($F_{(1,49)}$=8.44, p<.01). Furthermore, this ANOVA resulted in an emotion x group interaction ($F_{(2,98)}$=4.24, p<.05, GG-corr.) in which the clinical group gave lower ratings to fearful and neutral faces than the control group (p<.001 and p<.05 respectively). Finally, the ANOVA for the condition 'how wide is the nose?' resulted in a main effect for emotion ($F_{(2,98)}$=331.39, p<.001), with higher subjective scoring for happy faces than for fearful and neutral faces (both p's<.001). Also, subjective scoring was higher for fearful faces than for neutral faces (p<.001). There was no main effect of group or an interaction effect with group in this condition. See also figure 2 for an overview of the behavioral effects.

Reaction times

The ANOVA for reaction times resulted in a main effect for emotion ($F_{(2,98)}$=4.04, p<.05), with higher reaction times for fearful faces than for happy faces (p=.05).

Whole brain analyses

We first performed whole brain analyses to examine whether the task activated brain regions that were previously found to be related to emotional face processing. The whole brain Omnibus ANOVA for the positive effect of condition showed activation in bilateral amygdala, bilateral insula and bilateral prefrontal cortex (PFC; see also Figure 3a). To further investigate the task effect we created the contrasts fearful faces > fixation, happy faces > fixation and neutral faces > fixation (Figure 3b, c and d). These contrasts resulted in activation in (bilateral) amygdala, bilateral insula and bilateral PFC. Finally we created the contrasts fearful faces > neutral faces and happy faces > neutral faces. These contrasts resulted in activation in the bilateral amygdala, bilateral uncus and bilateral inferior frontal gyrus/insula for fearful faces > neutral faces and activation in the left amygdala, left insula and medial prefrontal cortex for happy faces > neutral faces (Figure 3e, f).

To examine whether the amygdala specifically was responsive to emotional valence and not to attention condition (as expected based on our previous study, van den Bulk et al., 2013), we also created the contrasts fear rating > passive viewing, happy rating > passive viewing, nose rating > passive viewing, fear rating > nose rating and happy rating > nose rating. The analyses showed that all active conditions resulted in more activation in bilateral PFC compared to the passive viewing condition. Importantly, amygdala activation was not modulated by attention condition.

Furthermore, we examined the main effect of group and the interaction effect between group and condition. These contrasts showed no significant patterns of brain activation for the main effect of group and for the interaction effect between group and condition, suggesting that there are no significant differences in whole brain activation between groups and for all conditions.

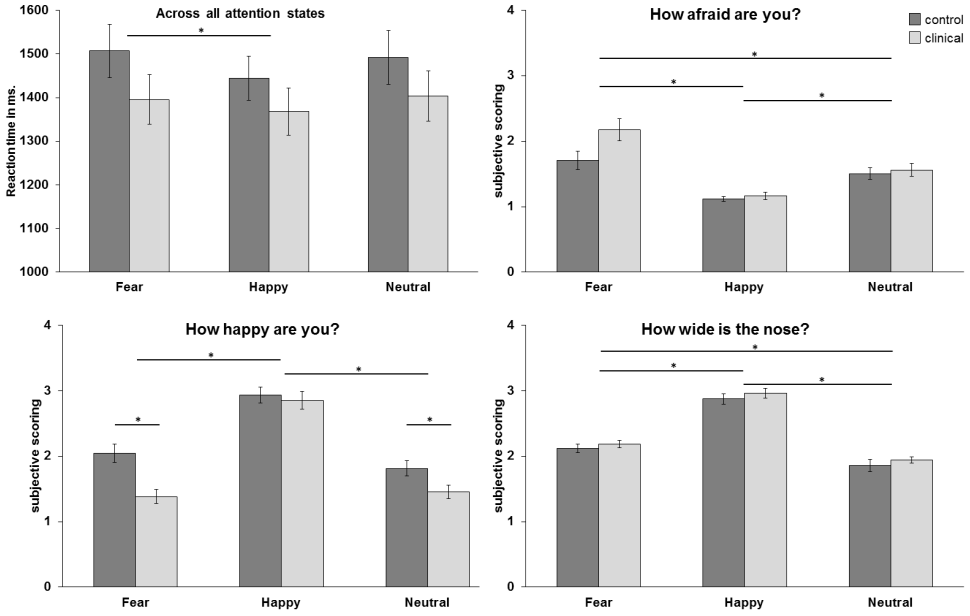

Figure 2. Mean reaction times in milliseconds across all attention conditions and mean subjective scoring per attention condition. **= p<.05*

Region of interest analyses

The analyses presented above were followed up by ROI analyses allowing us to detect smaller changes in specific regions that do not survive whole-brain comparisons, thereby allowing for a more detailed test of potential group differences. Results are reported for anatomically defined amygdala ROIs, based on the MNI templates available in SPM (see Figure 4). The percent signal change values of the left and right ROI were submitted to attention condition (4 levels) x emotion (3 levels) x group (2 levels) ANOVAs. Results were highly comparable for the masked functional amygdala ROIs, based on the contrast all faces > fixation, FDR corrected, p<.05, at least 10 continues voxels .

The ANOVA for left amygdala resulted in a main effect of emotion ($F_{(2,98)}$=10.09, p<.001, $\eta^2_{partial}$=.171). Post hoc analysis showed that amygdala

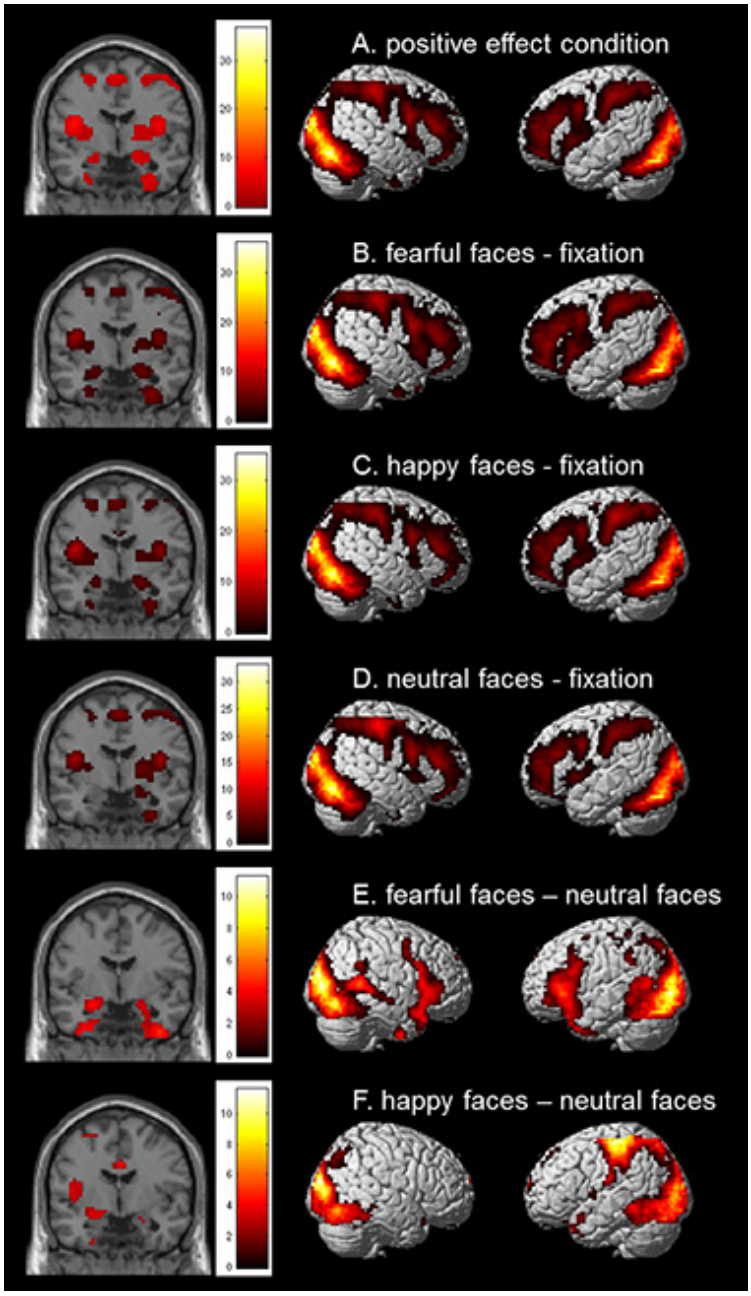

Figure 3. Whole brain contrast showing effects for A. positive effect of condition, B. fearful faces > fixation, C. happy faces > fixation, D. neutral faces > fixation, E. fearful faces > neutral faces and F. happy faces > neutral faces (N=51; FDR corrected, p<.05; 10 contiguous voxels). *MNI coordinate coronal slices: x = 21, y = -4, z = -17.*

was more active for fearful and happy faces than for neutral faces (resp. p=.001 and p<.01), while fearful and happy faces did not differ from each other (p=.40). Comparable results were obtained for the right amygdala, with a main effect of emotion ($F_{(2,98)}$=5.66, p=.005, $\eta^2_{partial}$=.104) resulting in more amygdala activation to fearful faces than to neutral faces (p<.05). There were no main or interaction effects with group. For both regions there was no main effect of group (left: $F_{(1,49)}$=.11, p=.74, $\eta^2_{partial}$=.002; right: $F_{(1,49)}$=1.57, p=.22, $\eta^2_{partial}$=.031), no interaction effect between group and attention condition (left: $F_{(3,147)}$=.62, p=.60, $\eta^2_{partial}$=.013; right: $F_{(3,147)}$=1.21, p=.31, $\eta^2_{partial}$=.024) and no interaction effect between group and emotion (left: $F_{(2,98)}$=.61, p=.54, $\eta^2_{partial}$=.012; right: $F_{(2,98)}$=.05, p=.95, $\eta^2_{partial}$=.001).

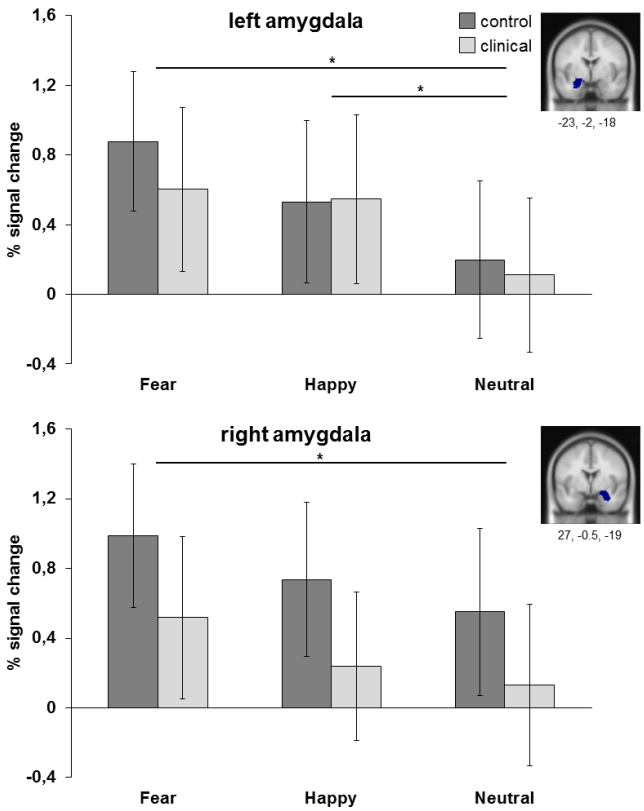

Figure 4. ROI analyses of left and right amygdala (anatomical). *Results are collapsed across attention conditions. *= p<.05*

Relation between depression/anxiety symptoms and amygdala activation

When correlating the percent signal change values of the amygdala ROIs (separately for fearful, happy and neutral faces relative to fixation) with anxiety (RCADS) and depression symptoms (CDI), we only found significant positive correlations between right amygdala activation and self-reported anxiety for fearful, happy and neutral faces relative to fixation in the clinical group (Figure 5). The significant correlations ranged between r=.49 and r=.54, all with p<.05 (see also supplemental Table 2.). We found no significant correlations for self-reported depression symptoms in the clinical group (all p's≥.10). Furthermore, we found no significant correlations between amygdala activation and anxiety or depression symptomatology for the complete sample (N=51; all p's≥.17) and the control group (all p's≥.21).

We performed three linear regression analyses for N=22 adolescents from the clinical group with percent signal change of the amygdala ROI (for fearful, happy and neutral relative to fixation separately) as dependent variable and demeaned anxiety score (RCADS), depression score (CDI score; both in model I) and the interaction between both (in model II) as independent variables. For the regression analyses in which we included amygdala activation when viewing fearful faces, the results showed that model I explained 29% of variance (R^2=.290, $F_{(2,21)}$=3.884, p<.05) and that the anxiety scores significantly predicted amygdala activation when viewing fearful faces (β=.509, p<.05). Model II, in which the interaction between anxiety and depression scores was included, did not result in an increase in explained variance and was not significant. When performing the same analyses with amygdala activation when viewing neutral faces as dependent variable, model I explained 29.5% of variance (R^2=.295, $F_{(2,21)}$=3.977, p<.05) and again the anxiety scores predicted amygdala activation (β=.499, p<.05). In the analysis for happy faces model I explained 23.9% of variance (R^2=.239, $F_{(2,21)}$=2.991, p=.074) which was at trend level and anxiety scores predicted amygdala activation (β=.480, p=.057).

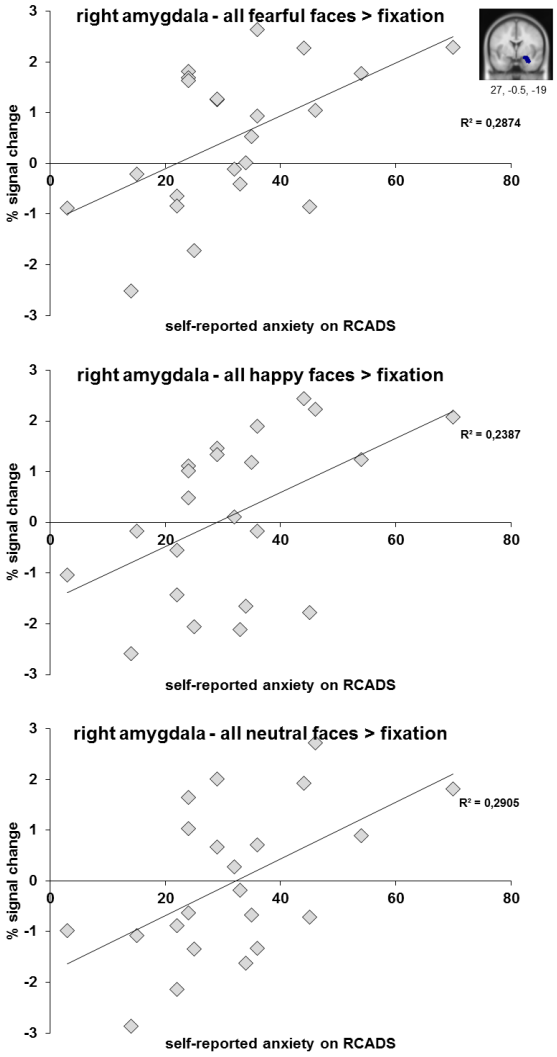

Figure 4. ROI analyses of left and right amygdala (anatomical). *Results are collapsed across attention conditions. *= p<.05*

Analyses reported were also performed while including age in years in step one and when excluding males. The results of these analyses were highly comparable with the results reported here.

Discussion

The objective of this study was to investigate emotional face processing in a sample of treatment naïve adolescents with a depression or anxiety diagnosis. In this sample with comorbid depression and anxiety symptoms we investigated the contribution of self-reported dimensional depression and anxiety scores to specific patterns of amygdala activation. The emotional face processing task activated expected brain regions in the emotional face processing network (e.g. bilateral amygdala, bilateral insula and bilateral PFC). Contrary to prior reports (Mcclure et al., 2007b; Monk et al., 2008a; Monk et al., 2008b; Roberson-Nay et al., 2006; Thomas et al., 2001a), we found no differences between the clinical group and the control group in the whole brain analyses or in the more specific ROI analyses of the amygdala. However, consistent with other prior studies (Thomas et al., 2001a) we found strong positive correlations within the clinical group between levels of self-reported anxiety symptoms and right amygdala activation, for all three types of emotional valence (i.e., fearful, happy and neutral face processing). Interestingly, there were no significant relations between amygdala activation and self-reported depression symptoms. Follow-up regression analyses confirmed that levels of self-reported anxiety were predictive for right amygdala activation. These correlation and regression effects were not present in the complete sample of N=51 and not in the control group. This suggests that the relation between self-reported anxiety symptomatology and amygdala activation may be specific for adolescents with depressive/anxiety disorders. Furthermore, it might indicate that there was not enough variance within the scores of the control group to find significant correlations.

The whole brain results showed bilateral amygdala activation to fearful, happy and neutral faces, with a stronger response to fearful and happy faces in the left amygdala and to fearful faces only in the right amygdala. These results correspond to the existing literature in which heightened patterns of amygdala activation are often reported when viewing negative emotional faces (Costafreda et al., 2008; Davis, & Whalen, 2001). Notably, researchers

have shown that the amygdala also responds to positive emotional faces (Fusar-Poli et al., 2009; Somerville et al., 2004; Van Den Bulk et al., 2013). The results of our study correspond to these findings and support prior conclusions that the amygdala is more of a general emotion processing node than only a negativity/fear processing node (Cunningham, Van Bavel, & Johnsen, 2008; Whalen, 1998).

Previous studies in which adolescents with a clinical depression or anxiety disorder were included, reported differentiating patterns of amygdala activation in the clinical group when they were compared with a control group (Mcclure et al., 2007b; Monk et al., 2008a; Monk et al., 2008b; Roberson-Nay et al., 2006; Thomas et al., 2001a). Yet, in the current study we could not replicate these results: the clinical adolescents did not show significantly differentiating patterns of amygdala activation on whole brain or ROI level. The absence of this effect was present in contrasts in which we used fixation as baseline condition and in which we used neutral faces as baseline condition. One of the reasons that we did not find group differences in amygdala activation may be due to the changes we made to the original task (Van Den Bulk et al., 2013) or differences in recruitment between studies. For example, we chose to include stimuli of fearful, happy and neutral facial expressions and not of angry and sad facial expressions. Also, we used direct gaze instead of averted gaze, only one head orientation (straight) and we asked the participants to focus on their own subjective experience during face viewing. It might be that the use of other task designs (e.g. rating of arousal or valence; see for an overview Costafreda et al., 2008; Sauer, Mothes-Lasch, Miltner, & Straube, 2013) results in different findings.

Furthermore, prior studies have differed by including adolescents with only a specific depressive or anxiety disorder. We included adolescents with various clinical diagnoses of affective disorders in our study, as we feel that taking a more dimensional approach is more ecologically valid given the frequent comorbidity between depressive and anxiety disorders and symptomatology (Essau, 2008). Furthermore, previous research indicated that de-

pression is often (72% of cases in community setting and 62% of cases in clinical setting) preceded by an anxiety disorder (Essau, 2008), which also highlights the tight relation between these disorders. By including a combined group and by taking the comorbidity of symptomatology into account we were able to investigate the specificity of the underlying mechanisms in both depressive and anxiety disorders. We think that this is a better approach to these clinical disorders.

Although we did not find a significant difference in amygdala activation between groups, we were still interested in the unique contribution of self-reported depression and anxiety symptoms to amygdala activation. That is to say, an individual difference analysis may be more sensitive for detecting heightened amygdala activation, as this may be present more in those adolescents with most severe problems. When taking the dimensional perspective of symptom severity into account, we found a significant positive relation between levels of self-reported anxiety and amygdala activation in the clinical group, which is in line with previous clinical studies (e.g. Thomas et al., 2001a). The current findings suggest that the level of anxiety symptoms, and not depression symptoms, seems to be a good predictor for differentiating patterns of amygdala activation independent of clinical disorder/diagnosis. This in turn might suggest that during adolescence anxiety symptoms and the relation with amygdala activation is an underlying trait characteristic for both depression and anxiety disorders and that depression symptoms are more a state characteristic. In the current study, adolescents who score high on anxiety symptomatology show more amygdala activation independent of emotional valence. It might be that these adolescents show a heightened vigilance in general and not only for scary or frightening situations. In future research this should be further investigated by, for example, also collecting data on personality traits like 'neuroticism' or by applying the state trait anxiety inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). Research already indicated that both state and trait anxiety highly relates to neuroticism (Del Barrio, Moreno-Rosset, Lopez-Martinez, &

Olmedo, 1997; Kotov, Gamez, Schmidt, & Watson, 2010) and it would be interesting to further investigate the relation between state and trait anxiety symptoms, neuroticism and differentiating patterns of amygdala activation. When translating the current findings to our understanding of the symptoms belonging to anxiety and when taking the absence of the effect in the control group into account, it might be that there is a predisposition for the development of depression or anxiety that may be expressed by personality styles like neuroticism. However, these ideas are highly speculative and further research is necessary to investigate this. For example, it would be interesting to see whether children/young adolescents who score high on neuroticism earlier in life have a higher chance of developing depression and/or anxiety during adolescence/young adulthood and to see whether this relates to differentiating patterns of amygdala activation.

There are some limitations in the current study that should be mentioned. First, even though the sample size of both our groups (N=25 clinical adolescents and N=26 controls) is relatively large compared to other studies (e.g. Monk et al., 2008a; Monk et al., 2008b; Thomas et al., 2001a) it might have been too small to find robust group differences. In addition, including a larger group of adolescents with clinical depression and anxiety disorders would be helpful to examine the relationship between anxiety and depression symptoms with amygdala activation in more detail. Therefore, future studies should aim for a more equal distribution between adolescents with DSM-IV depression and anxiety diagnosis and larger sample sizes to better isolate the relative contributions of depression and anxiety symptoms on a dimensional scale. Second, the age range of the participating adolescents was quite broad. Previous research has indicated that amygdala activation might be influenced by development, since children, adolescents and adults show different activation patterns when viewing emotional faces (Hare et al., 2008; Somerville, Fani, & Clure-Tone, 2011). Even though we did not find age effects in our study, future studies should include adolescents within a smaller age range and ideally use multiple adolescent groups with slightly

different ages to examine the developmental pattern of amygdala activation in both clinical and non-clinical adolescents. Within these future studies it would also be interesting to investigate the effect of puberty in relation to depressive and anxiety disorders, symptomatology and amygdala activation. Levels of progesterone, which relate to the menstrual cycle in females, influence patterns of amygdala activation during emotional face processing (Derntl et al., 2008). This probably also relates to puberty, and maybe even to depression or anxiety symptomatology. In the current study we did collect information about puberty stages (not about menstrual cycle), but the majority of adolescents already met post-puberty criteria and there was not enough variability within the sample to perform valid analyses. Finally, our sample included more female than male participants, which might have influenced our results. However, it is known that depressive and anxiety disorders are much more common in females than in males, which might underline the clinical validity of our sample. Nevertheless, it would be interesting to examine sex differences on the functioning of the amygdala related to face processing in future studies.

To conclude, the current study revealed that levels of self-reported anxiety were associated with patterns of amygdala activation for different types of emotional faces and across clinical diagnoses. Our findings thereby confirmed our hypothesis that anxiety symptoms are related to amygdala activity, but disconfirmed the hypothesis that clinical groups in general are different from healthy control participants. As such, a dimensional perspective seems to be a better approach for differentiating patterns of brain activation than categorical division of clinical versus non-clinical adolescents. In future research, it will be important to include longitudinal measurements to further investigate the relation between symptomatology, amygdala activation and treatment outcome in adolescents. Also, it would be interesting to include functional connectivity analyses to examine the relation between differentiating patterns of amygdala activation and connectivity with other brain regions, for example PFC. These suggestions are in line with the re-

search Domain Criteria approach in which a dimensional approach is considered important to advance understanding of mental disorders (Insel et al., 2010). Extending our knowledge on these topics, can give more information about individual differences in treatment outcome. The results of those studies can set the stage for the development of new diagnosis and treatment guidelines for adolescent depressive and anxiety disorders. This study is a first step in this process by highlighting the need for more research to better characterization of participant groups in future studies.

Supplemental material

Supplemental Table 1. Whole brain activation patterns for the contrasts: A. positive effect of condition, B. fearful faces > fixation, C. happy faces > fixation, D. neutral faces > fixation, E. fearful faces > neutral faces and F. happy faces > neutral faces. *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).*

Contrast	Region	Side	z-score	K_E	x	y	z	
A.								
All faces -fixation	Superior frontal gyrus	L	Inf.	846	0	20	49	*
	Superior frontal gyrus	L	Inf.		0	14	55	
	Superior frontal gyrus	L	2.52	14	-9	50	52	
	Superior frontal gyrus	L	2.33		-6	56	46	
	Middle frontal gyrus	L	7.18		-51	41	22	
	Middle frontal gyrus	L	6.71		-48	47	-8	
	Middle frontal gyrus	L	3.18	50	-24	-4	55	
	Precentral gyrus	L	2.78		-42	-10	64	
	Lingual Gyrus	L	Inf.		-18	-79	-14	
	Lingual Gyrus	R	Inf.	14111	12	-82	-8	*
	Middle occipital gyrus	R	Inf.		39	-73	-14	
	Cerebellar Tonsil	R	2.74	12	24	-37	-44	
	Cingulate gyrus	L	3.60	41	-3	2	28	
	Anterior cingulate	L	2.91	14	0	2	-11	
	Insula	L	Inf.	2937	-39	-4	16	*
	Thalamus	L	6.22	93	-18	-31	1	
	Substania Nigra	L	2.72		-9	-22	-8	
B.								
Fearful faces -fixation	Lingual gyrus	R	Inf.	12954	12	-82	-8	*
	Superior frontal gyrus	R	Inf.	819	3	14	55	*
	Superior frontal gyrus	L	Inf.		0	20	49	
	Middle frontal gyrus	L	7.41		-51	38	22	
	Middle frontal gyrus	L	6.94		-48	50	12	
	Middle frontal gyrus	L	3.15	35	-27	-4	58	
	Middle frontal gyrus	L	2.58		-39	2	61	
	Postcentral gyrus	L	6.04		-50	-22	52	
	Inferior parietal lobule	L	6.67	1218	-48	-31	46	*
	Inferior parietal lobule	L	5.98		-36	-42	45	
	Superior temporal gyrus	L	2.77	15	-42	17	-38	
	Lingual gyrus	L	Inf.		-18	-79	-14	
	Middle occipital gyrus	R	Inf.		39	-73	-14	
	Cingulate gyrus	L	2.83	10	-3	5	28	
	Insula	L	7.42	3241	-39	-4	16	*

	Thalamus	L	6.35	79	-21	-28	-2	
C.								
Happy faces - fixation	Superior frontal gyrus	L	2.52	10	-9	50	49	
	Middle frontal gyrus	L	6.83		-51	41	22	
	Lingual gyrus	L	Inf.		-3	-82	-5	
	Lingual gyrus	R	Inf.	14711	12	-82	-8	*
	Middle occipital gyrus	R	Inf.		39	-73	-14	
	Cerebellar tonsil	L	3.62	60	-30	-40	-35	
	Cingulate gyrus	L	4.32	75	-3	2	28	
	Cingulate gyrus	L	2.22		-12	-4	34	
	Anterior cingulate	L	3.77	27	0	2	-11	
	Anterior cingulate	L	2.47		-12	29	10	
	Insula	L	Inf.	2893	-39	-4	16	*
	Thalamus	L	6.44		-21	-28	-2	
D.								
Neutral faces - fixation	Superior frontal gyrus	L	7.60		0	11	58	
	Middle frontal gyrus	L	6.38		-51	41	22	
	Middle frontal gyrus	L	5.91		-48	47	-11	
	Middle frontal gyrus	L	2.79	13	-24	-4	55	
	Postcentral gyrus	L	5.91	1016	-51	-28	49	*
	Postcentral gyrus	L	5.46		-53	-22	52	
	Inferior parietal lobule	L	5.87		-45	-37	46	
	Lingual gyrus	L	Inf.		-18	-79	-14	
	Lingual gyrus	R	Inf.	11967	12	-82	-8	*
	Middle occipital gyrus	R	Inf.		39	-72	-14	
	Cerebellar tonsil	R	2.74	10	24	-37	-44	
	Cingulate gyrus	L	7.78	803	-3	17	46	*
	Cingulate gyrus	L	3.19	32	-6	5	28	
	Cingulate gyrus	L	2.47		-12	-1	31	
	Insula	L	7.40	2187	-39	-4	16	*
	Uncus	L	3.30	13	-30	-10	-35	
	Uncus	R	5.46	85	30	-7	-38	
	Thalamus	L	5.19	51	-18	-31	1	

Supplemental Table 2. Pearson correlations between anxiety (RCADS) and depression (CDI) subscale scores and parameter estimates of left and right amygdala ROI values for N=22 adolescents from the clinical group. **= p<.05, **= p≤.01*

	CDI depr.	**RCADS anx**
CDI - depr.	-	-
RCADS - anx	.534**	-
l amygdala fearful	.203	.167
l amygdala happy	.082	.112
l amygdala neutral	.330	.262
r amygdala fearful	.324	.537**
r amygdala happy	.274	.489*
r amygdala neutral	.342	.539**

Chapter 3

Habituation to emotional faces in depressed and anxious adolescents

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van den Bulk, B.G., Somerville, L.H., van Hoof, M.J., van Lang, N.D.J., van der Wee, N.J.A., Crone, E.A., Vermeiren, R.R.J.M.
Habituation effects during emotional face processing in adolescents with internalizing disorders, sexually abused adolescents and healthy controls.

Abstract

Adolescents with depressive and anxiety disorders and adolescents who experienced childhood sexual abuse show a large overlap in symptomatology. Research indicated hyper responsiveness and sustained activation instead of habituation of amygdala activation to emotional faces in adolescents with depressive and anxiety disorders and in adolescents who experienced childhood trauma. Little is known, however, about whether the same patterns of amygdala activation and habituation are present in these two groups. The current study examined habituation patterns of amygdala activity to emotional faces (fearful, happy and neutral) in adolescents with a DSM-IV depressive and/or anxiety disorder (N=25), adolescents who experienced childhood sexual abuse (CSA; N=19) and healthy controls (N=26). Behaviorally, adolescents with depressive/anxiety disorders and adolescents who experienced CSA reported more anxiety to fearful and neutral faces than controls. On whole brain level, there was a significant interaction between run and group within the left amygdala. ROI analyses showed elevated initial activity in the amygdala and rapid habituation in the CSA group compared to the depression/anxiety and healthy control group. These findings suggest that habituation patterns provide an additional index of emotional face processing problems, possibly showing that fearful responses in trauma groups habituate faster over time, whereas adolescents with depressive and anxiety disorders show less malleability.

Introduction

One of the most salient characteristics for social information processing is reading emotions from faces: multiple types of information, such as gender, age, emotional state and trustworthiness are processed within several hundred of milliseconds and provide crucial information for social interactions (Adolphs, 2002; Fusar-Poli et al., 2009; Grossmann, & Johnson, 2007). Prior research has shown that the fusiform cortex and the amygdala are important brain regions involved in this process (Fusar-Poli et al., 2009), where the amygdala is often interpreted as a region involved in detecting the valence and intensity of expressed emotions (Costafreda et al., 2008; Whalen et al., 2009). Developmental neuroimaging studies have reported that activity in this network is restructured in mid adolescence (Casey, Jones, & Somerville, 2011), such that intensified emotion-processing makes the amygdala especially sensitive to reading emotions from faces of unknown others (Scherf, Smyth, & Delgado, 2013). Several studies have reported that the amygdala shows stronger activity to emotional face processing in mid adolescence compared to childhood and adulthood (Guyer et al., 2008; Hare et al., 2008; Pfeifer et al., 2011; Somerville et al., 2011).

At the same time, there are pronounced individual differences in amygdala responsiveness in both adulthood and adolescence. Several reports have shown that responsiveness is higher in individuals who report higher levels of depression or anxiety or are diagnosed with one of these disorders (Monk et al., 2008b; Roberson-Nay et al., 2006; Somerville et al., 2004; Thomas et al., 2001a; Van Den Bulk et al., 2014), or who have a history of childhood maltreatment such as emotional, physical or sexual abuse (Garrett, Carrion, Kletter, Karchemskiy, Weems, & Reiss, 2012; Gee et al., 2013a; Hart, & Rubia, 2012). These findings suggest that reactivity of the amygdala may be more intense in individuals who report emotional problems. Individuals who experienced childhood maltreatment and who develop subsequent Post-Traumatic Stress Disorder (PTSD) are at increased risk to develop depressive and anxiety disorders over the course of life (Lindert, Von Ehrenstein, Gras-

how, Gal, Braehler, & Weisskopf, 2014). This, in combination with the comparable levels of increased amygdala activation in response to emotional faces (Hart, & Rubia, 2012; Monk et al., 2008a; Monk et al., 2008b; Roberson-Nay et al., 2006; Thomas et al., 2001a), highlights the need to investigate whether similar underlying neurobiological mechanisms are present in these groups. To our knowledge, there is no research published that directly compared the underlying higher amygdala responsiveness in adolescents with a depressive and/or anxiety disorder and adolescents who report emotional problems because of experiencing childhood sexual abuse (CSA). It is possible that there are neurobiological differences between these two groups: adolescents who experienced CSA have distinct characteristics like the experience of one or more traumatic events, which might have caused the activation of different underlying neurobiological mechanisms for depression and anxiety related symptoms.

Even though it is challenging to reveal differentiating neurobiological mechanisms between highly related clinical disorders, one way to examine whether the groups have different underlying response patterns is by studying habituation effects. In healthy populations, it is well known that the amygdala habituates to observed emotional expressions over time (Breiter et al., 1996; Fischer, Wright, Whalen, Mcinerney, Shin, & Rauch, 2003). The results of studies investigating habituation of amygdala activation in individuals with inhibited states, like depression and anxiety, are inconsistent. For example, a study by Hare and colleagues (2008) showed that adolescents with higher self-reported anxiety ratings habituated more slowly to observing emotional faces than adolescents with low levels of self-reported anxiety ratings. However, this study did not include information about the cause of heightened self-reported anxiety: it was not known whether it was related to childhood trauma or general patterns of anxiety independent of trauma. Two other studies reported relatively strong habituation effects during face processing within the amygdala in a sample of adults with social anxiety disorder (Sladky et al., 2012) and a sample of female students scoring high on

fear questionnaires (Wendt, Schmidt, Lotze, & Hamm, 2012). Again, no information on childhood trauma was available. To extend the current literature, it is of interest to compare amygdala habituation patterns in adolescents with depressive and/or anxiety disorders and adolescents who experienced childhood maltreatment, such as CSA.

In this study, we examined amygdala habituation in two groups that have previously been found to show elevated amygdala responsiveness to emotional faces. We included individuals with a DSM-IV diagnosis of a depressive or anxiety disorder, adolescents who experienced CSA, and a matched control group of adolescents without psychiatric complaints or traumatic experiences. Participants performed an emotional face-processing task validated in prior work (Monk et al., 2003a; Van Den Bulk et al., 2013; Van Den Bulk et al., 2014), and we reanalyzed the data for habituation patterns for subgroups of individuals by separating the task in three runs.

We aimed to test for dissociable habituation effects between groups based on the hypotheses that healthy control group participants will show fast habituation in the amygdala (Breiter et al., 1996), that both clinical groups will show increased amygdala activation in response to emotional faces (Garrett et al., 2012; Mcclure et al., 2007b; Roberson-Nay et al., 2006) and that the depression/anxiety group will show sustained activation in the amygdala (Hare et al., 2008). We were particularly interested in whether adolescents with CSA showed a similar pattern as adolescents with depression/anxiety without trauma, or whether their neural patterns were dissociable, suggesting that their anxiety and depression symptoms are related to a different underlying neural sensitivity.

Methods

Participants

Functional MRI data were collected based on 31 healthy controls, 30 treatment naïve adolescents with a clinical diagnosis of a current DSM-IV de-

pressive or anxiety disorder but no childhood trauma, and 22 adolescents who experienced childhood sexual abuse (CSA; comorbidity with anxiety and/or depression due to the CSA was allowed). Of the original sample, 12 adolescents were excluded for the current analyses due to various reasons: technical problems during scanning (N=4), excessive head movement (> 3mm.; N=5), unforeseen clinical features in the control group (N=1), or anomalous findings reported by the radiologist (N=2). The final sample consists of 26 healthy controls, 26 adolescents with a depressive or anxiety disorder and 19 adolescents with CSA (Table 1). All adolescents took part in the larger EPISCA study (Emotional Pathways' Imaging Study in Clinical Adolescents). The two clinical groups were scanned before the start of regular Cognitive Behavioral Therapy (CBT) based treatment.

Adolescents from the two clinical groups were recruited in outpatient departments of tree child and adolescent psychiatric institutes in Leiden and Haarlem. Inclusion criteria for participants in the depression/anxiety group were: having a clinical diagnosis of any DSM-IV depressive or anxiety disorder, no experience of CSA, being referred for regular CBT-like psychotherapy, and being treatment naïve. Inclusion criteria for the CSA group were: having lifetime experiences of sexual abuse by one or more perpetrators in- or outside the family and being referred for CBT-based therapy. Adolescents in the control group were recruited through local advertisements, 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 21 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 (except for stable SSRI use; N=4), current substance abuse, a history of neurological disorders or severe head injury, left-handedness, and general MRI contra-indications.

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, 1997). There was a significant difference between groups in age ($F_{(2,70)}$=4.02, p<.05) and IQ ($F_{(2,70)}$=3.63, p<.05), but not for sex distribution ($\chi^2_{(2,71)}$=.28, p=.87). The CSA group was significantly older and scored significantly lower on the IQ test than the control group (p<.05 and p<.05). The depression/anxiety group did not significantly differ from the control and CSA group (all p's>.10). For this reason, age and IQ were added as covariates in all subsequent analyses.

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.

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

	Depr./anx.	**CSA**	**Control**				
	N	**N**	**N**	χ^2	**df**	***p***	
N	26	19	26				
Females/Males	22/4	17/2	23/3	.282	2	.868	
	Mean$_{(sd)}$	**Mean$_{(sd)}$**	**Mean$_{(sd)}$**	**F**	**df**	***p***	
Age	15.98$_{(1.45)}$	16.62$_{(1.79)}$	15.25$_{(1.64)}$	4.02	2,70	.022	CSA>CNTR
Full scale IQ	105.12$_{(8.66)}$	99.89$_{(9.10)}$	106.58$_{(7.77)}$	3.63	2,70	.032	CNTR>CSA
DSM-IV depression/anxiety classification:	**N$_{(%)}$**	**N$_{(%)}$**	**N$_{(%)}$**				
No depressive/anxiety disorders	0	19$_{(100%)}$	26$_{(100%)}$				
Depression	7$_{(27%)}$	0	0				
Dysthymia	10$_{(38%)}$	0	0				
GAD	3$_{(11.5%)}$	0	0				
SAD	2$_{(8%)}$	0	0				
Anxiety NOS	1$_{(4%)}$	0	0				
Adjustment disorder with dep./anx.	3$_{(11.5%)}$	0	0				
DSM-IV PTSD classification:							
No PTSD	19$_{(73%)}$	1$_{(5%)}$	26$_{(100%)}$				
PTSD (sexual abuse)	0	16$_{(84%)}$	0				
PTSD (other cause)	7$_{(27%)}$	0	0				
PTSD (sexual abuse + other cause)	0	2$_{(11%)}$	0				
Self-reported symptomatology†	**Mean$_{(sd)}$**	**Mean$_{(sd)}$**	**Mean$_{(sd)}$**	**F$_{(group)}$**	**df**	***p***	
CDI: total score*	19.06$_{(9.10)}$	15.92$_{(7.12)}$	4.56$_{(3.40)}$	30.62	2,66	<.001	CLIN>CNTR
RCADS: total score anxiety subscales**	31.84$_{(14.16)}$	34.69$_{(14.36)}$	14.85$_{(10.83)}$	15.84	2,65	<.001	CLIN>CNTR
TSCC: total score***	42.51$_{(22.67)}$	44.31$_{(21.69)}$	17.63$_{(13.80)}$	12.99	2,64	<.001	CLIN>CNTR

*†Univariate ANOVA's for CDI, RCADS anxiety and TSCC were corrected for age and IQ; *=questionnaire data was missing for one participant of the depression/anxiety group and three participants of the CSA group; ***=questionnaire data was missing for three participants of the depression/anxiety group and two participants of the CSA group; ***=questionnaire data was missing for three participant of the depression/anxiety group and three participants of the CSA group; CNTR = control group, CLIN = depression/anxiety and CSA 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) were used to obtain DSM-IV-based classifications of depressive and anxiety disorders and Post Traumatic Stress Disorder (PTSD). Standardized dimensional measures were used for assessing the severity of self-reported symptoms of depression, anxiety and trauma; i.e. the total score of the Children's Depression Inventory (CDI) (Kovacs, 1992), the total anxiety scale of the Revised Children's Anxiety and Depression Scale (RCADS) (Chorpita et al., 2000) and the total score of the Trauma Symptom Checklist for Children (TSCC) (Briere, 1996). 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.

For clinical questionnaires, expectation maximization was used when items in the CDI (8 items across all participants), the RCADS (4 items across all participants) and the TSCC (6 items across all participants) were missing.

Task

All participants performed an emotional face-processing task, which was described in detail previously (Van Den Bulk et al., 2013; Van Den Bulk et al., 2014). In short, the task consisted of three randomly presented constrained ('how afraid are you?', 'how happy are you?' and 'how wide is the nose?') and one unconstrained (passive viewing) state questions. After state presentation, participants viewed 21 pictures expressing a fearful, neutral or happy face (a total of 21 trials per state question; 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. The task used a mixed design and different state questions were

included to divert attention towards or away from features of the face that provide information for emotion processing.

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 provided a rating to the probe question (Figure 1). Trials during which the participants did not respond within 3000 milliseconds (1.91% in total) were not included in the behavioral analyses and included as regressor of no interest in the fMRI analyses.

Since we were interested in habituation effects we modeled the three runs separately for the fMRI data. To be sure that enough trials were present per condition, we collapsed across state questions and only focused on emotional valence of the faces (fearful, happy and neutral), which results in 28 trials per condition per run. In prior studies, we found that amygdala activity was not influenced by state questions (van den Bulk et al., 2013; van den Bulk et al., 2014).

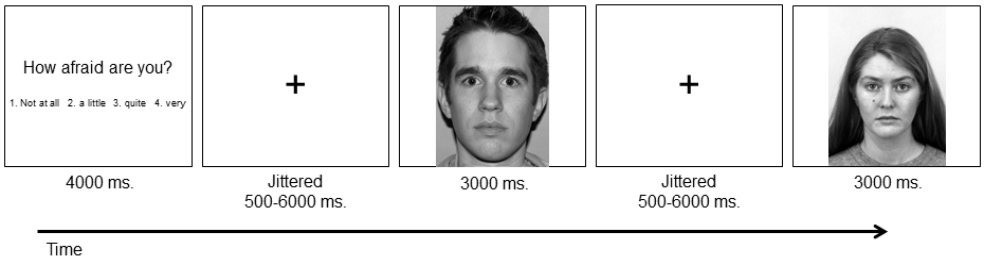

Figure 1. Visual representation of the emotional face-processing task. *Participants were first presented with one of the state questions (i.e., how happy are you, how afraid are you, how wide is the nose or passive viewing) followed by a fixation cross. Thereafter, twenrty-one pictures with a negative, positive or neutral face was shown (random selection) 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. First, a localizer

was obtained for each participant. Subsequently, 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. At the start each run had two additional volumes, which were discarded to allow for equilibration of T1 saturation effects. Also, a sagittal 3-dimensional gradient-echo T1-weighted image was acquired 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. Stimuli were presented onto a screen located at the head of the scanner bore and viewed by participants by means of a mirror mounted to the head coil assembly. Participants were able to indicate their ratings by using a button box, which was attached to their leg.

fMRI analyses

The collected data were analyzed using SPM8 (Welcome Department of Cognitive Neurology, London). Functional time series were realigned to compensate for small head movements and differences in slice timing acquisition. Functional volumes were first registered and normalized onto the individual structural T1 and thereafter to the T1 template. The normalization algorithm used a 12-parameter affine transformation together with a nonlinear transformation involving cosine basis functions and resampled the volumes to 3 mm cubic voxels. Functional volumes were spatially smoothed with an 8 mm, full-width at half-maximum isotropic Gaussian kernel. 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 were analyzed using the general linear model in SPM8. The fMRI time series were modeled by a series of events convolved with a canonical hemodynamic response function (HRF). The state questions were modeled separately as 4000 millisecond events as covariates

of no interest. The picture presentation of each emotional face was modeled as a zero duration event. In the model, the picture presentation was further divided in nine separate function trials (three runs by three expressed emotions). 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, the contrasts were computed by performing a full-factorial model with group as a three-level factor and treating subjects as a random effect. Task- and habituation related responses were considered significant if they consisted of at least 10 contiguous voxels at a FWE-corrected threshold of $p<.05$.

Based on the current literature on face processing and habituation we selected the amygdala as an a priori structure of interest to test our hypotheses on habituation. To analyze voxels within the amygdala we selected Regions Of Interest (ROIs) based on an unbiased contrast of all faces > fixation (N=71; FWE corrected, $p<.05$, at least 10 contiguous voxels), and we constrained the selection of active voxels to be within the anatomical boundaries of the amygdala using MarBaR in SPM8 (http://marsbar.sourceforge.net/; (Brett et al., 2002). This resulted in the right amygdala ROI. The left amygdala ROI was derived from the same contrast but with FDR instead of FWE correction, because it was not significantly active at this stringent threshold. The left amygdala ROI spanned several functional brain regions and therefore was subdivided by sequentially masking the functional ROI with the anatomical MarsBaR ROI. The percent signal change values (which were derived from the beta values) of the two ROIs were further analyzed using 3 (runs) x 3 (emotions) repeated measurement ANOVAs in SPSS 19 and all post-hoc comparisons were Bonferroni corrected.

Results

Behavioral data

Self-reported levels of depression, anxiety and trauma symptoms

The univariate ANOVA for self-reported levels of depression (CDI) resulted in a significant effect for group ($F_{(2,66)}$=30.62, p<.001) in which the depression/anxiety group and the CSA group scored significantly higher than the control group (both *p's*<.001). For the RCADS anxiety scale and the TSCC total scale comparable results were obtained: a significant effect of group ($F_{(2,65)}$=15.84, p<.001 and $F_{(2,64)}$=12.99, p<.001 respectively) in which the depression/anxiety group and the CSA group scored significantly higher than the control group (all *p's*<.001). On all scales, the depression/anxiety and CSA group did not differ from each other.

Subjective rating of emotional faces

For the subjective scoring of emotional faces three separate analyses with run (1-3) and emotion (fearful, happy, neutral) as within-subject factors and group as a between subjects factor were performed using repeated measurement ANOVAs in SPSS 19. The scores were analyzed separately for each state question, because values of the scores represent different interpretations for each question. In case sphericity could not be assumed, a Greenhouse-Geisser correction (GG-corr.) was used. Post-hoc comparisons were Bonferroni corrected.

The repeated measurement ANOVA for the state 'how afraid are you?' resulted in a main effect of group ($F_{(2,64)}$=4.19, p<.05) and an emotion x group interaction effect ($F_{(4,128)}$=3.29, p<.05). This interaction revealed that the adolescents with a depressive/anxiety disorder (p<.05) and the adolescents with CSA (p<.05) gave higher scores to fearful faces than the control adolescents. For happy and neutral faces there were no significant differences between groups (all *p's*>.10; see Figure 2).

The ANOVA for the state 'how happy are you?' resulted in a main effect of group ($F_{(2,62)}$=5.56, p<.01), but no group x emotion interaction. The

main effect of group showed that the overall subjective scoring of the control group was higher than for the depression/anxiety group (p<.01), whereas the CSA group did not differ from the anxiety/depression or the control group (both p's>.15).

Finally, the ANOVA for the state 'how wide is the nose?' resulted in a main effect of emotion ($F_{(2,130)}$=5.98, p<.005), with higher subjective scoring for happy and fearful faces compared to neutral faces (both p's<.001), and higher subjective scoring for happy than for fearful faces (p<.001). There was no main/interaction effect with group.

There was no main or interaction effect of run in any of the state questions suggesting an absence of habituation at the behavioral level.

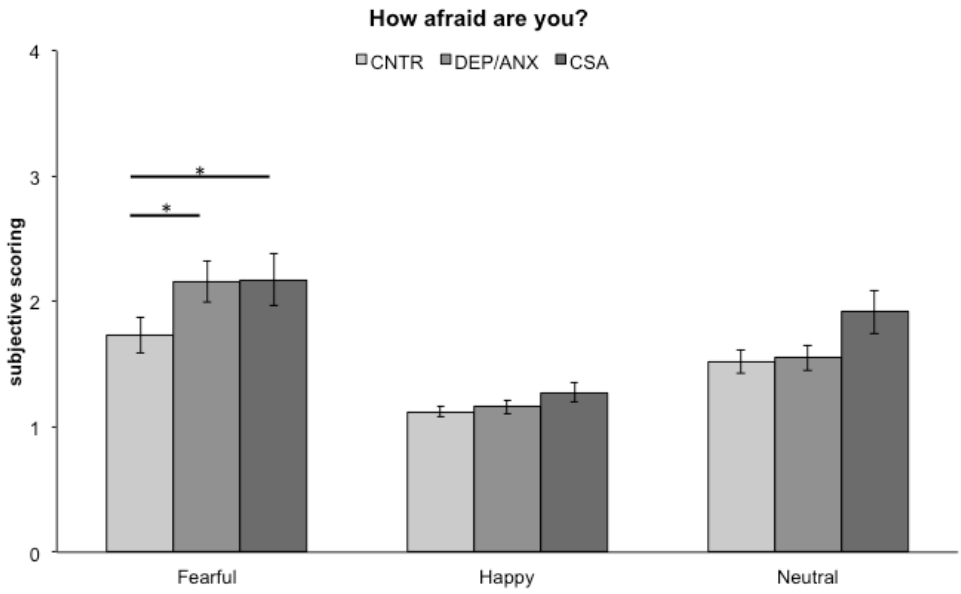

Figure 2. Group differences in subjective scoring of emotional faces with the 'How afraid are your?' attention state. *The two clinical groups reported being more afraid for fearful faces than the control group. * p < 0.05; CNTR=control group; DEP/ANX=depressed anx anxious adolescents; CSA=adolescents who experienced childhood sexual abuse.*

Whole brain analyses

The whole brain analysis for all faces > fixation resulted in robust activation in right amygdala and bilateral insula across participants (Figure 3A). The contrast run 1>run 3 resulted in significant activation in bilateral amygdala, suggesting changes in amygdala activation over time across participants. To follow-up the run effect, we inspected the main effect of group within the contrast run 1>run 3 (i.e., a group x time interaction). The results showed a significant group effect specifically in the left amygdala (uncorrected, p<.001, 10 voxels, no regions were detected when applying FDR or FWE correction; Figure 3B). Follow up t-tests for the contrast run 1>run 3 for each group separately revealed activation in this region only for the CSA group (p<.001 uncorrected). These findings suggest differences between groups in habituation patterns in the left amygdala when testing across the whole brain. The patterns across runs for the three groups were examined in detail using region of interest since region of interest analyses typically have more power to detect small group differences.

Region of interest analyses

Region of interest analyses were performed for the right and left amygdala in a run x emotion x group repeated measurement ANOVA. For right amygdala (Figure 4), the repeated measurement ANOVA resulted in a run x group interaction effect ($F_{(4,132)}$=2.62, p<.05). For the CSA group there was a significant decrease in activation between run 1 and run 2 (p<.05), between run 1 and run 3 (p<.001) and between run 2 and run 3 (p<.01). A comparable pattern of a decrease in amygdala activation was seen for the control group: run 1-run 2, p<.05 and run 1-run 3, p<.01. For the depression/anxiety group there were no significant in- or decreases in activation over runs (p's>.10). Furthermore, the CSA group showed significantly more amygdala activation in run 1 compared to the depression/anxiety group (p=.05). The three groups showed no significant differences in run 2 and run 3 (all p's>.10).

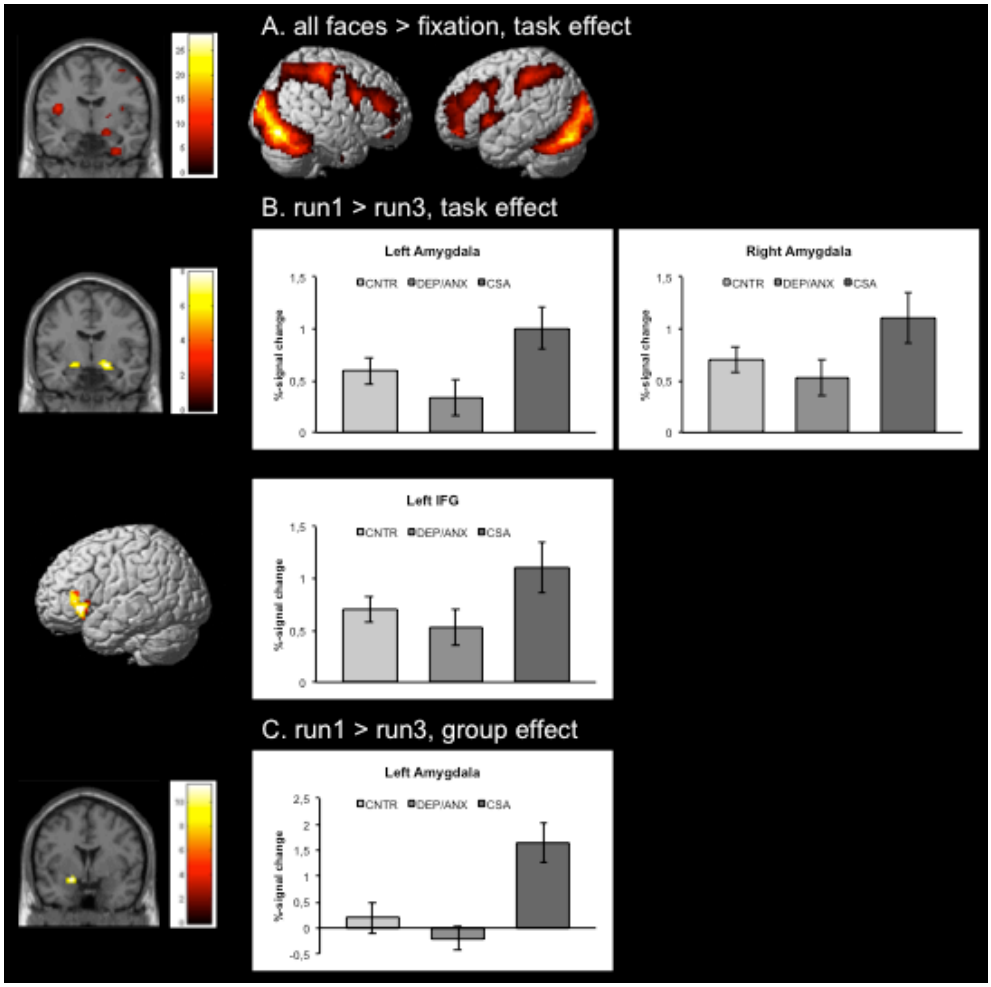

Figure 3. Overview of whole brain results derived from a full factorial model including three groups and three runs. *A. the contrast all emotional faces > fixation for the main effect of the task (FWE corrected, p<.05; 10 contiguous voxels), B. the contrast emotional faces in run 1 > emotional faces in run3 for the main effect of the task (FWE corrected, p<.05; 10 contiguous voxels) and C. the contrast emotional faces in run 1 > emotional faces in run3 for the main effect of group (uncorrected, p<.001; 10 contiguous voxels). Left and right amygdala and left inferior frontal cortex (represented in B and C) were followed up by ROI analyses to visualize the direction of the effects. CNTR=control group; DEP/ANX=depressed anx anxious adolescents; CSA=adolescents who experienced childhood sexual abuse.*

The same analysis for the left amygdala also resulted in a run x group interaction ($F_{(4,130)}$=3.85, p=.005). The CSA group showed a significant decrease in activation between run 1 and run 2 (p=.001) and between run 1 and run 3 (p<.001). For the control group there was a significant decrease in acti-

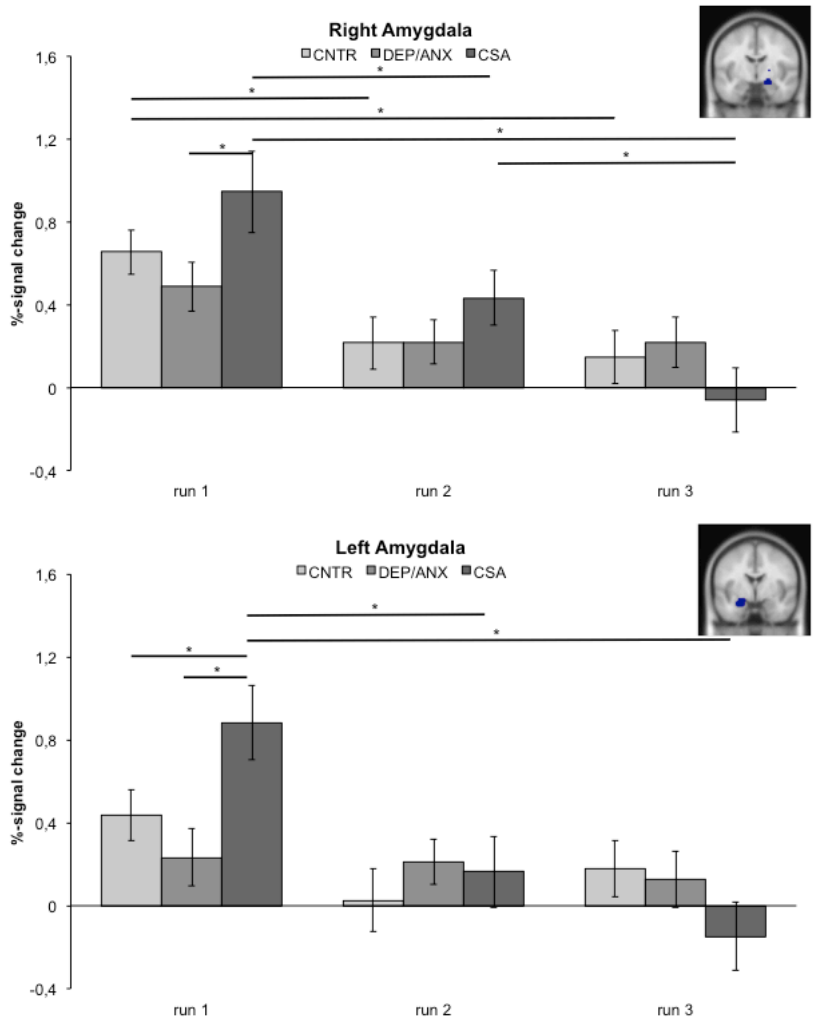

Figure 4. Region of interest analyses for left and right amygdala. *Regions were derived from the contrast all emotional faces > fixation with a FEW correction for right amygdala (p<.05; 10 contiguous voxels) and a FDR correction for left amygdala p<.05; 10 contiguous voxels). * p < 0.05; CNTR=control group; DEP/ANX=depressed anx anxious adolescents; CSA=adolescents who experienced childhood sexual abuse.*

vation between run 1 and run 2 (p=.05). Again, within the depression/anxiety group there was no habituation effect (p's=1.00). Also, the CSA group showed significantly more activation in run 1 compared to both the depression/anxiety group (p=.001) and the control group (p<.05). There was no significant difference between the depression/anxiety group and the control group in run 1 and the three groups did not significantly differ from each other in run 2 and run 3 (all p's>.10).

To summarize, the results for both right and left amygdala showed elevated initial activity and rapid habituation of the amygdala in the CSA group when compared to the depression/anxiety group in which no habituation was detected. Overall, no significant main/interaction effects were found for facial expression (all p's>.10), suggesting that these effects were consistent across facial expressions.

Discussion

The goal of this study was to examine whether amygdala habituation during an emotional face-processing task differed between adolescents with a DSM-IV diagnosis of depression and/or anxiety disorder, adolescents who experienced CSA and healthy controls. This is important since depressed/anxious adolescents and adolescents with CSA not only show a large overlap in symptomatology (Lindert et al., 2014), but they also show distinct characteristics: adolescents who experienced CSA per definition experienced one or more traumatic events that might have influenced the development of different neurobiological mechanisms.

Consistent with prior studies (Breiter et al., 1996; Fischer et al., 2003), healthy adolescents showed a habituation effect in the amygdala (especially right) when viewing emotional faces: activation in right and left amygdala was significantly higher during run 1 than during run 2/run 3. This effect was present for all emotional faces, so not solely for fearful faces, which is in line with results of previous studies (Breiter et al., 1996). This suggests that habi-

tuation to (emotional) faces may be a general pattern that is related to, for example, the novelty of the emotional faces which adapts over time. Previous research already showed that right amygdala response for novel neutral faces is larger than for familiar neutral faces, but in both cases amygdala activation declined over time. Therefore, Schwartz and colleagues (2003) suggest that one function of the amygdala is to detect new events that might be important.

Within the clinical groups, habituation-related amygdala activity showed different patterns. For the CSA group we found initial increased activation in the amygdala and relatively fast habituation of amygdala activation to a level comparable to that of the depression/anxiety and control groups. In the depression/anxiety group we did not find significant habituation effects in the amygdala. Instead, the adolescents with depressive and/or anxiety disorders showed comparable levels of amygdala activation as the control group but showed no significant decline in amygdala activation over the three runs. The analyses partially confirmed our hypothesis: the control group showed a habituation effect in the amygdala while the depression/anxiety group did not show this effect. In addition, the results showed a difference between the two clinical groups in amygdala activation in which the CSA group had a higher initial response to emotional faces at the start of the task and showed faster habituation compared to the depression/anxiety group.

Contrary to prior reports (Mcclure et al., 2007b; Monk et al., 2008a; Monk et al., 2008b; Thomas et al., 2001a), the depression/anxiety group did not show a general higher amygdala response to emotional faces than the healthy adolescents. This finding was surprising, however, we previously reported that self-reported levels of anxiety and not diagnosis per se predicted amygdala activation (Van Den Bulk et al., 2014). Possibly, individual differences in depression and anxiety symptomatology suppressed group differences in amygdala activation. Another explanation can be found in the current task design: we used a task design in which participants rated their subjective feeling while some studies have shown that attention load (such

as answering questions or rating the emotional faces) influences amygdala activation (Costafreda et al., 2008; Sauer et al., 2013). We did include a passive viewing condition. However, not enough trials were left to examine habituation during passive viewing. Furthermore, passive viewing was preceded and followed-up by the other conditions and it is not clear to what extend attention load effects continue to be present. Future research should further investigate this by, for example, using a passive viewing task with a sufficient number of trials per run. Group differences may then be more pronounced.

The innovative aspect of the current study was that we included both adolescents with depressive/anxiety disorders and adolescents who experienced CSA. Although the overlap in reported symptomatology between the two clinical groups is high, CSA has an additional component namely the experience of one or more traumatic events. Previous research has indicated that people who experienced childhood maltreatment show heightened patterns of amygdala activation (Hart, & Rubia, 2012; Van Harmelen et al., 2013) and that experiencing childhood maltreatment often leads to the development of depressive and/or anxiety disorders, including PTSD (Lindert et al., 2014). With respect to the behavioral data (subjective scoring of emotional faces), we showed that adolescents who experienced CSA report the same elevated level of fear to fearful faces as depressed/anxious adolescents. However, at neurobiological level adolescents with CSA showed higher amygdala activation compared to healthy and depressed/anxious adolescents at the beginning of the task, but similar activation as controls near the end of the task. Possibly, the absence of habituation effects at behavioral level points at a discrepancy between what an individual feels and what is happing in the brain. This possibly relates to the theory of sustained fear levels not being effectively regulated by cognitive control regions (e.g. top-down regulation by the medial prefrontal cortex (PFC) (Mayberg, 1997).

Although speculative, there is a potential interpretation for the different habituation effects between depressed/anxious adolescents and adolescents with CSA. It might be that the depression and anxiety symptoms

reported by adolescents with CSA correspond with increased vigilance to emotional stimuli, which may result in increased amygdala activation in response to emotional faces. However, the down-regulation of this heightened amygdala response might be intact, resulting in habituation over time. In depressed and anxious adolescents a different mechanism might underlie their symptomatology: the primary emotional response is less exaggerated and maybe the integration of information by cognitive control regions is insufficient causing emotion regulation problems. More research is necessary to support this suggestion, for example by using two different paradigms (passive viewing task and emotion regulation task) and by conducting functional connectivity analyses. Within the current task design, it was not possible to conduct functional connectivity analyses because of the relatively fast event-related design and the many conditions.

Even though we aimed to include a comprehensive sample with a well-validated experimental task, several limitations of this study need to be mentioned. First, we had to collapse across state questions within the emotional face-processing task to have enough power left for the habituation analyses. This limits the ability to isolate specific task effects and possibly suppressed current findings. Future research could optimize this by using a task design specifically developed to investigate habituation effects in the brain. For example, by using a 'pure' passive viewing task including positive and negative emotional faces in which participants only have to indicate the gender of the actor expressing the emotion. This would decrease the influence of attention load on amygdala activation (Costafreda et al., 2008; Sauer et al., 2013). Another limitation is the significant difference in age and IQ between the control group and the CSA group. Although we controlled for age and IQ in all analyses, results might have be influenced by these differences. Future research should include participants within smaller age ranges who are matched on gender and IQ. It would also be interesting to include several age ranges within adolescence to investigate developmental differences between and within groups, since previous research has indicated that there are rela-

tively large developmental changes within the face processing network which includes the amygdala (Hare et al., 2008; Scherf et al., 2012).

Taken together, this study indicated that depressed/anxious adolescents showed different patterns of amygdala activation and habituation to emotional faces than adolescents with CSA. These findings inform our understanding of individual differences in adolescence by showing that adolescents with similar symptomatology but with different diagnosis can also show different patterns of habituation to emotional face stimuli. Possibly this can be helpful to improve intervention and treatment strategies: if replicated across samples, the results may indicate that it is potentially more helpful to focus on reducing the primary emotional responses in CSA and to focus on top-down regulation in depressed and anxious adolescents.

Chapter 4

Test re-test reliability of amygdala, prefrontal cortex and occipital cortex activation

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Abstract

Prior developmental functional Magnetic Resonance Imaging (fMRI) studies have demonstrated elevated activation patterns in the amygdala and prefrontal cortex (PFC) in response to viewing emotional faces. As adolescence is a time of substantial variability in mood and emotional responsiveness, the stability of activation patterns could be fluctuating over time. In the current study, 27 healthy adolescents (age: 12-19 years) were scanned three times over a period of six months (mean test-retest interval of three months; final samples *N*=27, *N*=22, *N*=18). At each session, participants performed the same emotional faces task. At first measurement the presentation of emotional faces resulted in heightened activation in bilateral amygdala, bilateral lateral PFC and visual areas including the fusiform face area. Average activation did not differ across test-sessions over time, indicating that at the group level activation patterns in this network do not vary significantly over time. However, using the Intraclass Correlation Coefficient (ICC), fMRI reliability demonstrated only fair reliability for PFC (ICC=0.41-0.59) and poor reliability for the amygdala (ICC<0.4). These findings suggest substantial variability of brain activity over time and may have implications for studies investigating the influence of treatment effects on changes in neural levels in adolescents with psychiatric disorders.

Introduction

Processing of emotional faces has consistently been associated with activation in the amygdala and prefrontal cortex (PFC). Therefore, both brain areas are considered part of the social information processing network and the overlapping face processing network (Scherf et al., 2012). These networks are known to be involved in the fast recognition of social stimuli (including faces), and the processing and the interpretation of social-affective stimuli (Adolphs, Tranel, & Damasio, 2003). Interestingly, prior studies have shown stronger activation in the amygdala when seeing fearful compared to happy or neutral faces (Costafreda et al., 2008), although increased activation for happy faces has been reported as well (Fusar-Poli et al., 2009; Somerville et al., 2004). In addition, studies have indicated that the PFC is more activated during explicit face processing compared to implicit face processing and that PFC is differentially activated depending on the context, such as whether the faces need to be rated or need to be passively viewed (Fusar-Poli et al., 2009; Monk et al., 2003b; Monk, 2008).

Pronounced differences in amygdala and PFC activation have been found across adolescent development (Casey et al., 2011). This is not surprising, because adolescence is a developmental phase characterized by ongoing changes in gray and white matter across the brain (Giedd et al., 1999), which is also related to enhanced plasticity in cognitive and emotional functioning (Steinberg, 2005): intensification of emotions (Dahl, 2004) and developmental improvements in face processing (Scherf, Luna, Avidan, & Behrmann, 2011).For example, when using an emotional go-nogo task with fearful and neutral faces, Hare et al. (2008) indicated that adolescents have exaggerated amygdala activation to fearful faces relative to children and adults. These findings are consistent with other studies reporting heightened amygdala responses to emotional faces in adolescence (Baird et al., 1999; Guyer et al., 2008; Monk et al., 2003b; Pfeifer et al., 2011; Thomas et al., 2001b). At present, most of these studies used cross sectional designs and therefore it is not yet known to what extent amygdala and PFC activation vary across time

during adolescence. There are only a few studies that used longitudinal study designs to investigate the processing of emotions in adolescents (Moore, Pfeifer, Masten, Mazziotta, Iacoboni, & Dapretto, 2012; Pfeifer et al., 2011; Shaw, Grosbras, Leonard, Pike, & Paus, 2011; Shaw, Grosbras, Leonard, Pike, & Paus, 2012). For example, a study by Pfeifer et al. (2011) investigated the neuronal coupling between ventral striatum and amygdala over time. However, none of these studies investigated the test-retest reliability of specific activation patterns. It is important to investigate the stability of brain activation patterns because it is closely related to the investigation of ongoing changes in gray and white matter and plasticity of the brain during adolescence. When we have more knowledge about stability of brain activation patterns, studies investigating plasticity can take this knowledge into account when interpreting their results, especially in studies investigating intervention effects.

Functional neuroimaging techniques are being investigated because of their potential for quantifying longitudinal brain activation changes associated with disease or intervention effects (Maslowsky et al., 2010; Mcclure et al., 2007a; Strawn, Wehry, Delbello, Rynn, & Strakowski, 2012b). In such repeated measures designs it is important to know whether brain activation patterns in healthy comparison subjects vary over time or not. When there is a lot of variation over time within healthy comparison subjects, this should be taken into account when performing longitudinal analyses in clinical samples investigating for example treatment effects. For this reason, test-retest reliability and reproducibility of fMRI over time are extensively studied in adults (for review, see Bennett, & Miller, 2010). So far, it is known that reliability varies depending on scan-interval, task and experimental design, method to assess reliability and sample characteristics (e.g. healthy vs. illness, young vs. old). The majority of reliability studies have focused on motor and cognitive tasks, with only few studies examining face processing. For example, Plichta et al. (2012) used an emotional face-processing task in which participants had to match a target stimulus (i.e. emotional face or geometric

shape) with one of two other stimuli (one corresponding to the target and the other being different). Their results indicated that amygdala activation showed good reliability on between-group level but poor reliability on within-subjects level (Intraclass Correlation Coefficient-values (ICC) values < .4). In a passive viewing face-processing task with neutral, happy and fearful facial expressions, poor to excellent ICC-values were reported depending on the contrast chosen (Johnstone et al., 2005). So far, none of these studies included adolescent participants, even though significant changes in emotional functioning occur during this stage of development. Therefore, the main goal of this study was to examine the variability of activation in the amygdala and PFC across multiple measurements in healthy mid-adolescents.

Previously, Monk and coworkers suggested that amygdala and PFC activation differs depending on the question that is posed prior to the presentation of the face. They demonstrated higher neural activation in several brain areas when adolescents had to rate emotions compared to when attending to a non-emotional feature of the face or during passive viewing (Mcclure et al., 2007b). However, how state questions influence the activation patterns of the amygdala and PFC is not yet well understood. By including state questions in the current paradigm, we were able to further investigate neural responses to emotional faces that are modulated by three different state questions and a passive viewing condition.

To test the questions posed in this experiment, we performed a longitudinal study in which healthy adolescents were scanned three times over a period of six months. During each scan session participants performed an adapted version of the face attention paradigm used in the studies of McClure and Monk and colleagues (Mcclure et al., 2007b; Monk et al., 2003b). We investigated neural responses to emotional faces and whether there were interactions with context. Based on these previous studies, we expected increased activation in bilateral amygdala, PFC and visual cortex. Furthermore, we expected higher test-retest reliability for the visual cortex and prefrontal cortex than for the amygdala (Plichta et al., 2012).

Methods

Participants

In total, 31 healthy right-handed adolescents (aged 12-19) participated in the first measurement of the fMRI experiment. They took part in the larger EPISCA study (Emotional Pathways' Imaging Study in Clinical Adolescents), a longitudinal MRI study in which adolescents (healthy comparison group and two clinical groups) were followed over a period of six months. Five of the 31 adolescents were excluded due to excessive head movement (> 4 mm; N=1), technical problems during scanning (N=1), an anomalous finding reported by the radiologist (N=1) or subclinical scores on some questionnaires (N=1), leading to a final sample of 27 adolescents for the first measurement (Mean Age=14.56, SD=1.60, 24 female). The samples for the longitudinal test-retest analyses consisted of 22 adolescents (two measurements; Mean Age at Time Point 1 (TP1)=14.45, SD=1.37, 19 females) and 18 adolescents (three measurements; Mean Age at TP 1=14.33, SD=1.37, 17 females). Estimated full scale IQ scores were acquired with the use of six subtests of either the Wechsler Intelligence scale for Children-III or the Wechsler Adult Intelligence Scale (Wechsler, 1991; Wechsler, 1997): picture completion, similarities, picture arrangement, arithmetic, block design and comprehension. All participants scored in the average range (TP1 (N=27) Mean=106, SD=7.4; TP1 (N=22) Mean=107, SD=7.3; TP1 (N=18) Mean=106, SD=7.7). The sex distribution was unequal with a higher number of females than males.

Adolescents were recruited through local advertisement. They were included if they met the following criteria: right-handed, normal or corrected-to-normal vision, sufficient understanding of the Dutch language, no history of neurological or psychiatric impairments and no contraindications for MRI testing. Furthermore, both parents and the adolescents were assessed with a semi-structured diagnostic interview (ADIS-C/P, Silverman, & Albano, 1996), and filled out several questionnaires (i.e., CBCL and YSR, Achenbach, 1991a; Achenbach, 1991b), to make sure that they did not have psychiatric problems. Informed consent was obtained by participants, and by parents

and participants in case of minors. The adolescents received a financial compensation including travel expenses for participation. The study was approved by the medical ethics committee of the Leiden University Medical Center. All anatomical scans were reviewed and cleared by a radiologist.

Procedure

All adolescents included in the study were scanned three times: first measurement (TP1), second measurement approximately three months after TP1 (TP2; $Mean_{(SD)}$=3.3 $months_{(0.43)}$) and third measurement, approximately six months after TP1 (TP3; Mean (SD)=6.6 months (0.63). At each measurement all participants were tested individually and were trained to lie still in a mock scanner, which simulated the environment and sounds of an actual MRI scanner. In-between scanning, participants were asked to report subjective stress levels on a visual analogue scale (VAS) ranging from 0-100 (Mean (SD) reported stress level: TP1 (N=27)=17.7 (14.5) range: 0-58.3, TP1 (N=18)=18.7 (16.3) range: 0-58.3, TP2 (N=18)=10.2 (12.4) range: 0-43.3, TP3 (N=18)=6.9 (7.9) range: 0-22.3. There was a significant decline in subjective stress level between measurements ($F_{(2,34)}$=8.4, p=.005), but no significant differences between subsamples at TP1. Stimulus presentation and the timing of all stimuli and response events were acquired using E-Prime software. Head motion was restricted by a pillow and foam inserts that surrounded the head.

Task

We administered a well-known face-attention paradigm (Mcclure et al., 2007b; Monk et al., 2003b) with a few adjustments: 1) angry faces were excluded (due to similar response of angry and sad in prior studies); 2) the state question 'how happy are you?' was included; 3) the number of response options was restricted from five to four; and 4) the number of trials was extended to get a good estimation of the BOLD response (Blood Oxygen Level Dependent). The adapted task consisted of three constrained conditions

(state questions: how afraid are you?; how happy are you?; how wide is the nose?) and an unconstrained state condition (passive viewing). States were rated for all faces on a four-point rating scale: (1) 'Not at all', (2) 'A little', (3) 'Quite' and (4) 'Very'. During the task, reaction times and subjective scoring of the different emotional faces were recorded for behavioral analyses.

The faces with emotional expressions were drawn from two widely used sets of standardized faces: (Karolinska (Lundqvist, Flykt, & Ohman, 1998) and NimStim faces (Tottenham et al., 2009b)) and were selected to resemble the Dutch population (equal amount of males/females and ethnic diversity). In total, 42 actors were selected who expressed fourteen fearful, fourteen happy and fourteen neutral faces. We are aware of the ongoing debate whether "neutral" faces exist, or whether "ambiguous" faces should be used (e.g. Tahmasebi et al., 2012), but for consistency we use the term 'neutral' faces.

Trials had the following structure: participants were presented with a state question for 4000 milliseconds, followed by a centrally located cue with a jittered interval between 500 and 6000 milliseconds, after which one of the pictures was shown for 3000 milliseconds followed by a centrally located cue with a jittered interval between 500 and 6000 milliseconds (Figure 1). During picture presentation, participants had to rate the picture by pressing one of four buttons. In case they did not respond within 3000 milliseconds, nothing happened and the next trial was presented. Missing trials (1.98% in total) were not included in the analyses. In total there were three runs consisting of four blocks, with each block representing one state. Each state was followed by 21 faces, with seven faces for each emotion (fearful, happy and neutral). The states were presented randomly and the pictures of faces with emotional expressions within a state were pseudo-randomly presented. In total there were 84 trials per run (four states * 21 faces), 63 trials per state (three runs * 21 faces), 84 trials per emotion (three runs * four states * 7 faces per emotion), 21 trials per condition (one of the state questions * one of the emotions) and 252 trials in total.

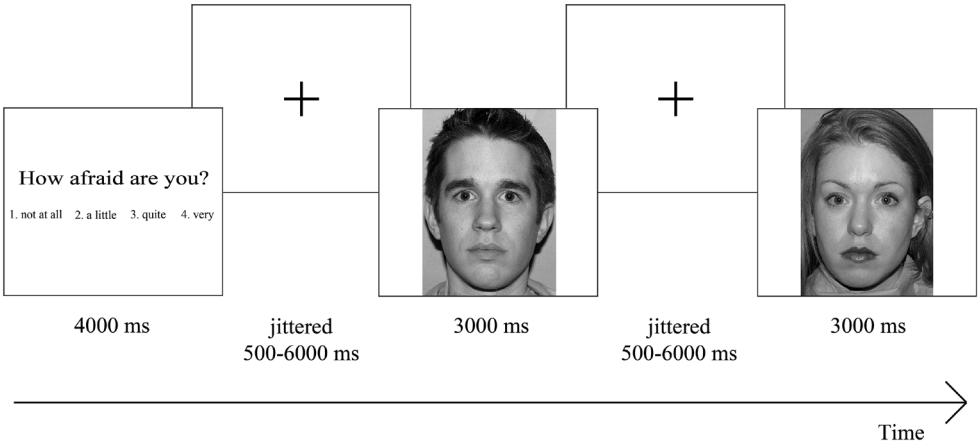

Figure 1. Display of task design. *Subjects were presented with one of four states, followed by a centrally located cue, after which one of the emotional faces was shown. Subjects were asked to rate each emotional face on a four-point rating scale ranging from 'not at all' to 'very', based on the presented state. During scanning reaction times and subjective scoring were registered.*

Image acquisition

Data were acquired using a 3.0T Philips Achieva (Philips, Best, The Netherlands) scanner at the Leiden University Medical Center. Stimuli were presented onto a screen located at the head of the scanner bore and viewed by participants by means of a mirror mounted to the head coil assembly. First a localizer was obtained for each participant. Subsequently, T2*-weighted Echo-Planar Images (EPI) (TR=2.2s. TE=30ms, 80 x 80 matrix, FOV=220, 38 slices of thickness 2.75 mm) were obtained during three functional runs of 192 volumes each. Each run had two additional scans at the start, which were discarded to allow for equilibration of T1 saturation effects. Also, a sagittal 3-dimensional gradient-echo T1-weighted image was acquired with the following scan parameters: repetition time 9 ms; echo time 3.5 ms; flip angle 80°; 170 sagittal slices; no slice gap; field of view 256 x 256 mm; 1 mm isotropic voxels.

fMRI analyses

The collected data were analyzed using SPM5 (Wellcome Department of Cognitive Neurology, London). The functional time series were realigned

to compensate for small head movements and differences in slice timing acquisition. Functional volumes were spatially normalized to the EPI template. 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. Functional volumes were spatially smoothed with an 8 mm, full-width at half-maximum isotropic Gaussian kernel. 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 were analyzed using the general linear model in SPM5. The fMRI time series were modeled by a series of events convolved with a canonical hemodynamic response function (HRF). The state questions were modeled separately as 4 sec events and were added as covariates of no interest. The picture presentation of each emotional face was modeled as a zero duration event. In the model, the picture presentation was further divided in twelve separate function trials (four state questions by three expressed emotions). 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 (i.e. all faces vs. fixation, fearful faces vs. fixation, happy faces vs. fixation and neutral faces vs. fixation). The resulting contrast images, computed on a subject-by-subject basis, were submitted to group analyses. At the group level, contrasts between conditions were computed by performing one-tailed *t*-tests on these images, treating subjects as a random effect. Task-related responses were considered significant if they consisted of at least 10 contiguous voxels at a corrected threshold of $p<.05$ (FDR corrected). Furthermore, we performed voxelwise ANOVAs to identify regions that showed time-related differences in relation to the picture presentation.

We used the MarsBaR toolbox for use with SPM5 (http://marsbar.

sourceforge.net/; Brett et al., 2002) to perform region of interest (ROI) analyses to further investigate patterns of activation. ROIs were defined based on a priori hypothesis and regions that were identified in the functional mask of the whole-brain analyses (all faces vs. fixation and happy faces vs. fixation; FDR corrected, $p<.05$, at least 10 contiguous voxels). ROIs used for the longitudinal analyses were based on the full baseline sample, i.e. N=27. ROIs that spanned several functional brain regions were subdivided by sequentially masking the functional ROI with each of several anatomical MarsBaR ROIs.

To analyze the reliability of brain activation we calculated Intraclass Correlation Coefficients (ICCs). To analyze the reliability of behavioral data and brain activation we calculated IntraClass Correlation Coefficients (ICCs). For the behavioral data (reaction times, subjective scoring and reported stress-level) we used stability analyses in SPSS ($ICC_{(3,3)}$). We calculated the ICC value for different conditions and different time point comparisons. Furthermore, we calculated measures of intra-voxel reliability on individual contrast values for each ROI by using the ICC toolbox provided by (Caceres, Hall, Zelaya, Williams, & Mehta, 2009). For this analysis, the same ROIs were used as for the functional analyses, i.e. based on the full baseline sample of N=27. Furthermore, we added bilateral inferior occipital regions to control for method validity, as these regions are associated with face processing (Plichta et al., 2012) and had substantial overlap with the functional ROIs derived from the all vs. fixation contrast (overlap: Left 56%, Right: 88%). By analyzing only ROIs based on the first measurement we could test whether the level of group activation of the first session could predict the consistency of activation within participants. Previous studies proposed different criteria regarding reliability criteria for fMRI studies. We followed the guidelines proposed by Cicchetti for qualifying reliability: poor (<0.4), fair (0.41–0.59), good (0.60–0.74) or excellent (>0.75) (Cicchetti, & Sparrow, 1981; Cicchetti, 2001). These proposed criteria parallel suggested acceptance levels of the neuroimaging community of critical ICC-values of 0.4 (Aron, Gluck, & Poldrack, 2006; Eaton et al., 2008).

Results

Behavioral data

Figure 2 shows the rating- and reaction time patterns for the full baseline sample (N=27). The results at TP1 were similar for those participants who took part in two (N=22) or three (N=18) follow-up measurements. For both subsets, there were no main effects for time.

Subjective rating of emotional faces

Time (3 levels) and emotion (3 levels) were added to the analysis as within-subject variables. The scores were analyzed separately for each state question, because values of the scores represent different interpretations for each state. In case sphericity was not assumed, Greenhouse-Geisser correction (GG-corr.) was applied.

The repeated measure ANOVAs resulted in main effects for emotion in all three states. In the 'how afraid are you?' state the main effect of emotion ($F_{(2,52)}$=13.27, p<.001) resulted in higher subjective scores for fearful and neutral faces than for happy faces (both *p's*<.005). For 'how happy are you?' the main effect of emotion ($F_{(2,52)}$=35.87, p<.001, GG-corr.) resulted in higher scores for happy faces than for both other faces (both *p's*<.001). Finally, in the state 'How wide is the nose?' state the main effect of emotion ($F_{(2,52)}$=174.13, p<.001) resulted in scores that were highest for happy faces and lowest for neutral faces (all *p's*<.001).

Reaction times

For reaction time, one repeated measure ANOVA was performed with a three (state) by three (emotion) design. The results showed a main effect for state ($F_{(2,52)}$=5.04, p<.05), a main effect for emotion ($F_{(2,52)}$=4.49, p<.05) and an interaction effect of state by emotion ($F_{(4,104)}$=4.44, p<.05). Reaction times were longer for the 'how happy are you?' state compared to the 'how afraid are you?' state (p<.01) and when viewing fearful faces compared to happy faces (p<.05).

Separate comparisons for each state resulted in a main effect of emotion ($F_{(2,52)}$= 9.32, p<.001) for the 'how afraid are you?' state, with longer reaction times for fearful (p<.005) and neutral (p<.05) faces than for happy faces. For the other two states ('how happy are you?' and 'how wide is the nose?'), no significant differences were found. Separate comparisons for each emotion resulted in a main effect for state ($F_{(2,52)}$=11.70, p<.001) for the happy faces, with longer reaction times for happy faces in the 'how happy are you?' (p<.005) and 'how wide is the nose?' (p<.005) states than in the 'how afraid are you?' state. For the separate comparison of neutral faces there was a main effect of state ($F_{(2,52)}$=3.95, p<.05), but none of the emotions differed from each other when further testing the main effect. For the fearful faces, no significant differences between states in reaction times were found.

For the subsets of N=22 (TP1-TP2) and N=18 (TP1-TP3) the analyses of subjective scores and reaction times showed similar results at TP1 and there were no significant main or interaction effects for time, indicating that these patterns were consistent over time and across state questions.

Test-retest reliability of behavioral data

To investigate the test-retest reliability of the behavioral data, we performed reliability analyses with the use of SPSS ($ICC_{(3,3)}$). We examined the different state questions, the emotional faces that were presented and the reported stress-levels. The results showed good ICC values for the subjective scoring of fearful faces for TP1-TP3 (ICC=.62). All other comparisons for both the reaction times and the subjective scoring resulted in excellent ICC values (ranging from .76to .96). Furthermore, the ICC values for the VAS-scores were poor for TP1-TP3 (ICC=.35), fair TP1-TP2 (ICC=.52) and TP1-TP2-TP3 (ICC=.69), but excellent for TP2-TP3 (ICC=.89)

fMRI analyses

The fMRI results are organized in three sections. First, neural responses to emotional faces and state questions were investigated in the sample

of 27 adolescents who participated in the cross-sectional part. Second, the effect of repeated task assessments was investigated for 22 adolescents who participated in two sessions and 18 adolescents who took part in three sessions with the use of ROI analyses. Third, the test-retest reliability was tested using ICCs.

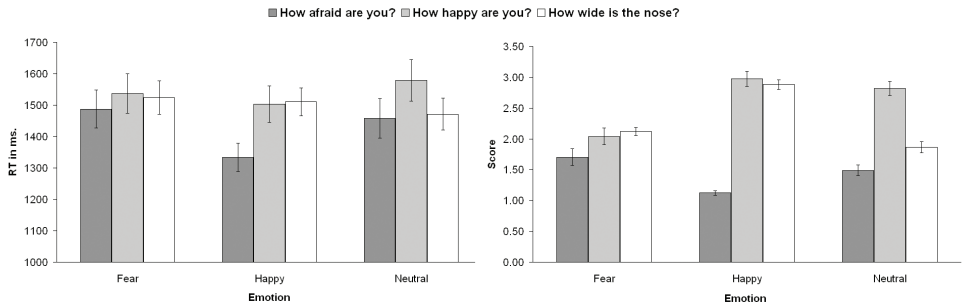

Figure 2. Mean reaction times and subjective scores for the different states and emotions *(N=27 at TP1).*

First measurement cross-sectional analyses: Effects of emotions and state questions

The neural responses to emotions were assessed by whole brain analyses in 27 adolescents. For this purpose, we ran four contrasts on the whole-brain level to extract ROIs for specific state question analyses (FDR corrected, $p<.05$, at least 10 contiguous voxels). The first contrast, all emotional faces > fixation, resulted in expected bilateral activation in the amygdala and bilateral activation in the lateral PFC (Figure 3a). The second contrast, fearful faces > fixation resulted in activation in bilateral amygdala and bilateral lateral PFC (Figure 3b). The third contrast, happy faces > fixation resulted in activation in bilateral amygdala and medial prefrontal cortex (Figure 3c). The final contrast, neutral faces > fixation, resulted only in bilateral lateral PFC activation (Figure 3d). Supplementary Table 1 lists the MNI coordinates for peak values of each activated region.

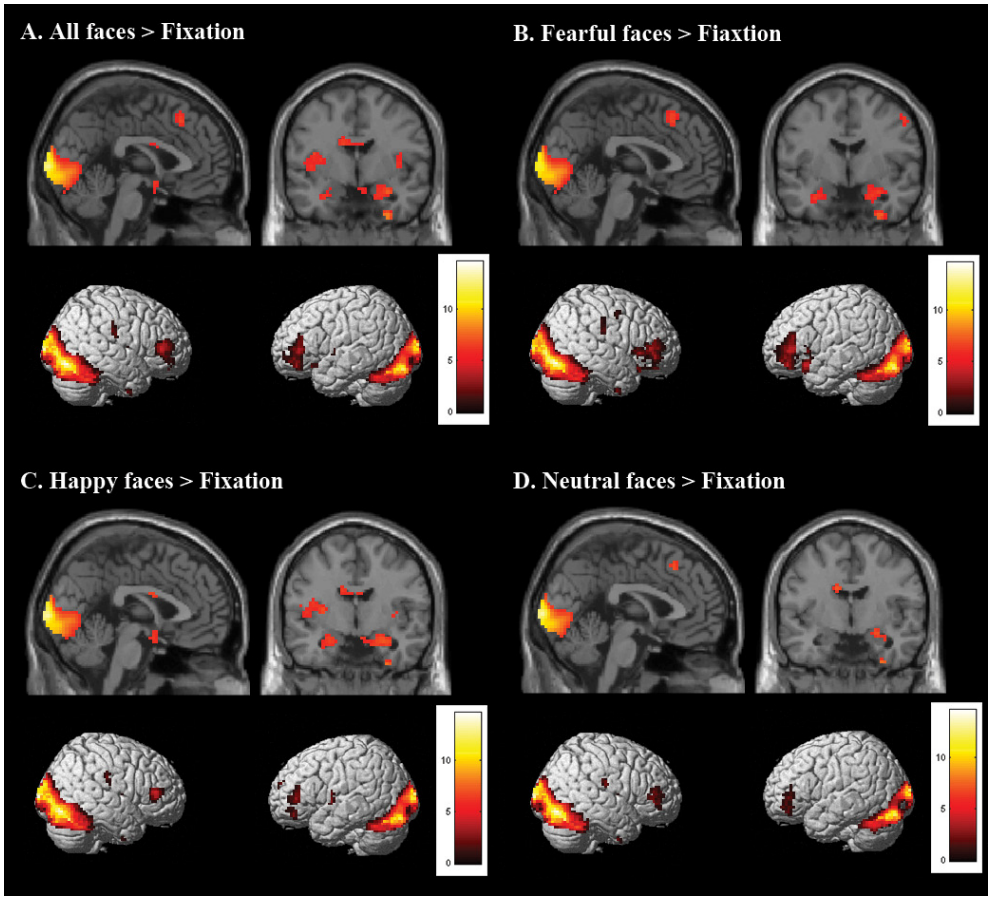

Figure 3. Stimulus-onset-locked whole brain contrast for N=27 at TP 1 showing effects of A. all faces > fixation, B. all fearful faces > fixation, C. all happy faces > fixation and D. all neutral faces > fixation (FDR corrected, p<.05; 10 contiguous voxels).

Next, three areas (amygdala, lateral PFC and medial PFC) were further explored in ROI analyses. Here we focus on left-lateralized areas due to space limitations. The ROI results of right-lateralized areas were highly comparable concerning main effects for emotion and state. All ROIs were defined based on task activation in the whole brain functional mask.The ROI for masked left amygdala (based on all faces > fixation) resulted in a main effect of emotion ($F_{(2,52)}$=3.20, p<.05). Post hoc comparisons showed that the amygdala responses were larger for happy than for neutral faces (p=.05), whereas fearful fa-

ces did not differ significantly from either happy or neutral faces. No main or interaction effects were found for the different states.

A main effect for emotion was found for left lateral PFC (based on all faces > fixation; $F_{(2,52)}$=6.11, p<.005). As expected, left lateral PFC was more active in response to fearful faces compared to both neutral and happy faces (both p's<.005). As depicted in Figure 4, left lateral PFC also showed a main effect of state ($F_{(3, 78)}$=6.65, p<.001). Post hoc comparisons revealed that left lateral PFC was less active following the question 'passive viewing' compared to 'how happy are you?' and 'how wide is the nose?' (p<.001 and p<.05 resp.).

Finally, a state effect was found in medial prefrontal cortex (based on happy faces > fixation; $F_{(3,78)}$=4.82, p<.01) showing that there was more activation following the question 'how afraid are you?' compared to 'passive viewing' (p<.05). Furthermore, there was more activation after the question 'how happy are you' compared to the question 'how wide is the nose?' (p<.05).

Taken together, prefrontal cortex (lateral and medial) was responsive to the state question, irrespective of emotional content, suggesting that this area is more sensitive to the specific context of the experiment than the amygdala.

Longitudinal analyses: testing for effects of time

All analyses reported above were repeated with the subsamples of 22 participants who took part in two measurements and of 18 participants who participated in all three measurements. As results were similar for both groups, we report the results of participants included in all three measurements.

We performed a whole brain repeated measures ANOVA (full factorial design) with time as an additional factor (i.e. testing for interactions between emotion, state and time). These analyses resulted in a highly comparable set of activation compared to TP1. Again, the contrast all emotions > fixation resulted in activation in bilateral amygdala and bilateral PFC (Figure 5a). The contrast fearful faces > fixation resulted in activation in bilateral PFC (Figure

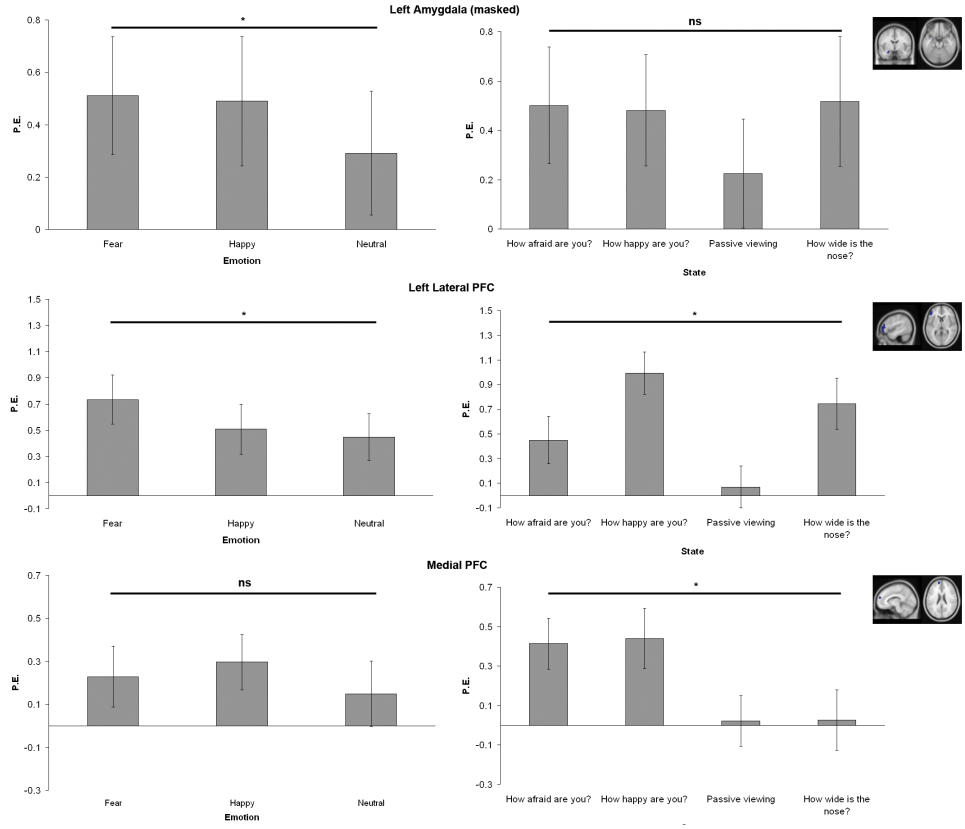

Figure 4. Parameter estimates for three ROIs: left amygdala (masked; based on all faces > fixation), left lateral PFC (based on all faces > fixation) and medial PFC (based on all happy faces > fixation). *Results are presented separately for the states and emotion (FDR corrected, p<.05; 10 contiguous voxels) and correspond to the sample of N=27 at TP1.*

5b) and the contrast happy faces > fixation resulted in bilateral amygdala and bilateral PFC activation (Figure 5c). Finally, the contrast neutral faces > fixation resulted in bilateral PFC and right amygdala activation (Figure 5d). The effects confirm the findings from the ROI analysis in the first measurement, which showed that the amygdala is more responsive to happy faces. Supplementary Table 2 lists the MNI coordinates for peak values of each activated region.

To investigate the effect of time we performed four repeated measures ANOVA using the flexible factorial design (one for each contrast). In

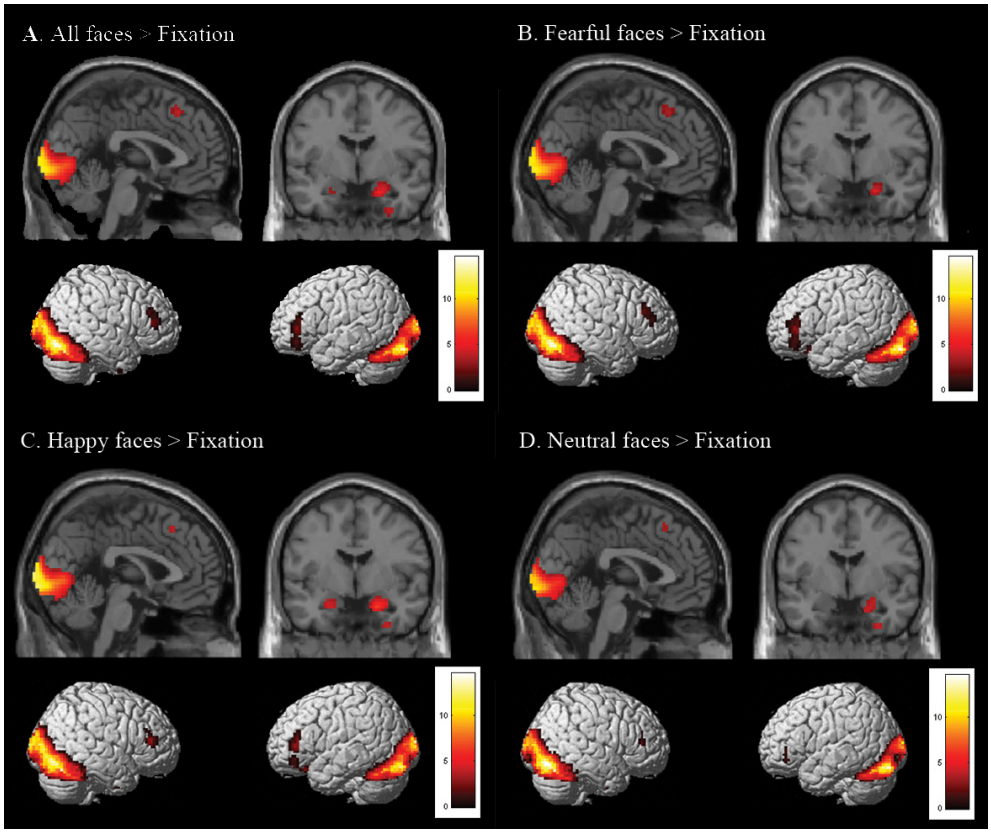

Figure 5. Stimulus-onset-locked whole brain contrast for N=18 at TP 1/TP2/TP3 showing effects of A. all faces > fixation, B. all fearful faces > fixation, C. all happy faces > fixation and D. all neutral faces > fixation (FDR corrected, p<.05; 10 contiguous voxels). *Results derive from a repeated measurement analysis in which time was taken as an additional factor.*

the analysis we included 'subjects' (independency=yes, variance=equal) and 'time' (independency=no, variance=equal) as factors. None of the analyses showed a main effect for time. The absence of this effect may suggest that, on group-level, the activations in these areas do not significantly vary over time. This was further tested using two approaches: (1) ROI analyses testing for time effects, because ROIs can have possibly more power for detecting small changes, and (2) test-retest reliability to test for stability within individuals.

ROI analyses testing for effects of time

As depicted in Figure 6, the time (3 levels) by state (4 levels) by emotion (3 levels) ANOVA for left amygdala resulted in a main effect of emotion (all faces > fixation; $F_{(2,34)}$=3.89, p<.05), but no interaction effect with time.

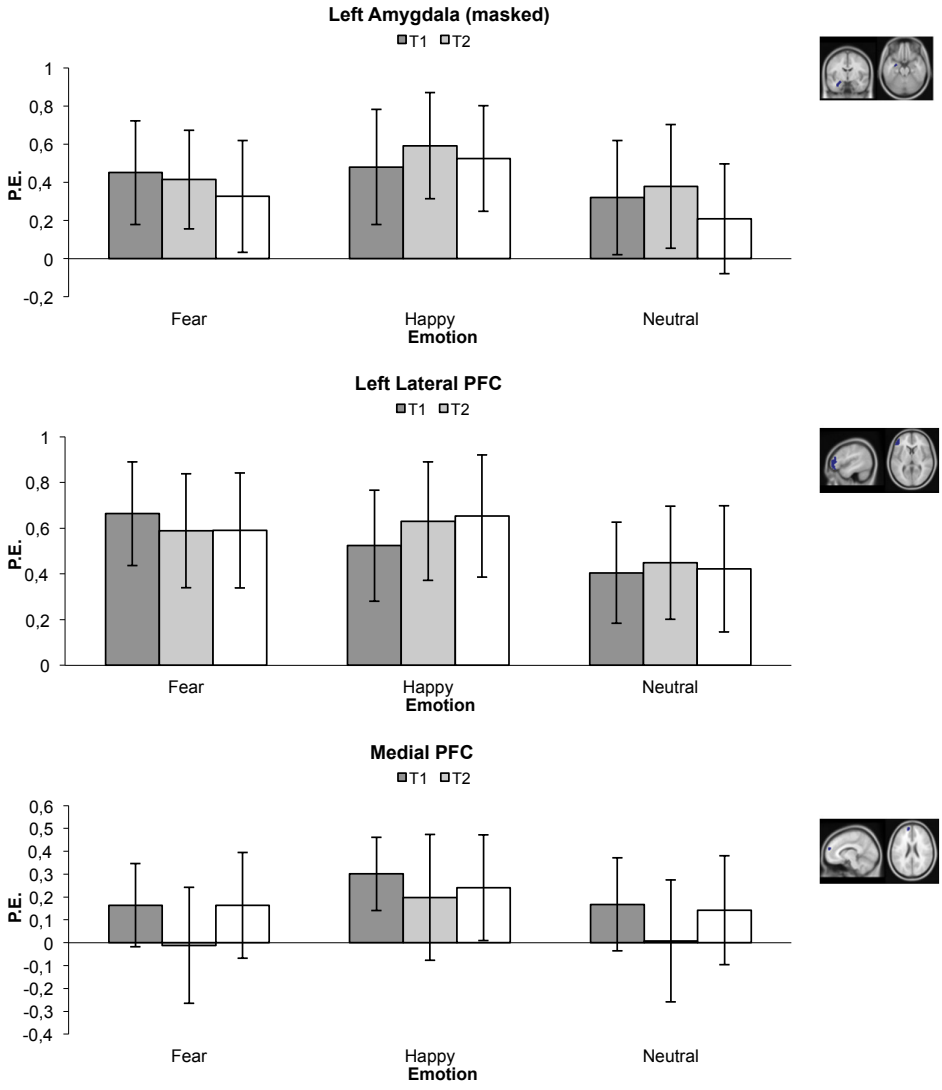

Figure 6. Parameter estimates for three ROIs: left amygdala (masked; based on all faces > fixation), left lateral PFC (based on all faces > fixation) and medial PFC (based on all happy faces > fixation). *Results are presented separately for the states and emotion (FDR corrected, p<.05; 10 contiguous voxels) and correspond to the sample of N=18 at TP1/TP2/TP3.*

Post hoc comparisons showed more activation for happy faces compared to neutral faces (p<.05). There was no difference in activation between fearful and happy faces and there was no main effect for state or time.

The time (3 levels) by state (4 levels) by emotion (3 levels) ANOVA for left lateral PFC resulted in a main effect for state ($F_{(3,51)}$=7.58, p<.001) and a main effect of emotion ($F_{(2,34)}$=5.09, p<.05), but no interaction effect with time. Specific post hoc comparisons for the main effect of state revealed that there was more activation in the condition 'How happy are you?' compared to the 'Passive viewing' and 'How afraid are you?' conditions (both p's <.05). Also, there was more activation in the condition 'how wide is the nose?' compared to 'how afraid are you?' (p<.05). Furthermore, the post hoc comparisons for the main effect of emotion showed more activation for fearful faces than for neutral faces (p<.05).

The time (3 levels) by state (4 levels) by emotion (3 levels) ANOVA for medial PFC resulted in a main effect for emotion, $F_{(2,34)}$=3.31, p=.05. Post hoc comparisons for this effect revealed more activation after happy faces compared to fearful faces (p=.05). No effects for state and/or time were found.

Test-retest reliability

Intra-voxel reliability (ICC) measures were based on ROIs at TP1 for the full baseline sample of adolescents (N=27; FDR corrected, p<.05, 10 contiguous voxels). Beside the functional ROI definition, anatomical and masked ROI were also defined and the results of these were highly comparable to the results of the functional ROIs (see supplemental table 3). Intra-voxel reliabilities were calculated for each contrast of interest and for each time period, resulting in ICCs for adolescents N=22 for TP1-TP2; and adolescents N=18 for TP1-TP2, TP2-TP3 and TP1-TP3. Table 1 list the ICCs for the contrast all emotions > fixation for each Time point. ICC-values for the other contrasts can be found in Supplementary Table 3a,b,c. Figure 7 displays ICC-values with SE bands for the TP1-TP2 (N=18) sample. ICCs were computed for each participant and the population estimate was based on bootstrap methods. Overall,

ICC-values were poor for the amygdala and right lateral PFC (<0.4), irrespective of Time Point and type of ROI (i.e. functional, masked or anatomical). Left lateral PFC demonstrated fair ICC-values [0.4-0.6] predominantly in the "all emotions" and "happy faces" >fixation contrast, while values varied between poor to fair in the other contrasts. The two "control" regions, i.e. bilateral inferior occipital cortices, showed excellent ICC-values for all, except one, contrasts and Time Points. Furthermore, the ICC-values for the different Time Point comparisons (TP1-TP2, TP1-TP3 and TP2-TP3) were similar, suggesting that the ICC-values were not influenced by the different scan interval.

Table 1. Reliability measurements of ROIs for all emotions vs. fixation.

All-Fix	TP1-TP2 N22	TP1-TP2 N18	TP2-TP3 N18	TP1-TP3 N18
ROI	medICC (SE)	medICC (SE)	medICC (SE)	medICC (SE)
L Amygdala Anat	0.07 (0.11)	0.10 (0.14)	0.28 (0.05)	0.21 (0.08)
L Amygdala	0.12 (0.12)	0.14 (0.13)	0.09 (0.12)	0.12 (0.07)
L Amygdala Masked	0.01 (0.20)	0.06 (0.23)	-0.02 (0.10)	0.10 (0.11)
R Amygdala Anat	0.15 (0.10)	0.15 (0.10)	0.34 (0.14)	0.34 (0.09)
R Amygdala	0.01 (0.14)	0.11 (0.18)	0.17 (0.10)	0.19 (0.07)
R Amygdala Masked	0.13 (0.14)	0.15 (0.15)	0.32 (0.14)	0.35 (0.12)
L LPFC	0.50 (0.12)	0.48 (0.13)	0.44 (0.07)	0.41 (0.08)
L LPFC InfTri Masked	0.50 (0.12)	0.45 (0.14)	0.56 (0.10)	0.36 (0.15)
R LPFC	0.35 (0.09)	0.32 (0.09)	0.31 (0.12)	0.28 (0.07)
R LPFC InfTri Masked	0.36 (0.10)	0.35 (0.11)	0.34 (0.12)	0.28 (0.11)
MedPFC	0.23 (0.14)	0.30 (0.12)	0.17 (0.10)	0.31 (0.19)
L Occipital Inf	0.84 (0.04)	0.81 (0.05)	0.86 (0.05)	0.85 (0.03)
R Occipital Inf	0.89 (0.03)	0.89 (0.03)	0.90 (0.04)	0.91 (0.03)

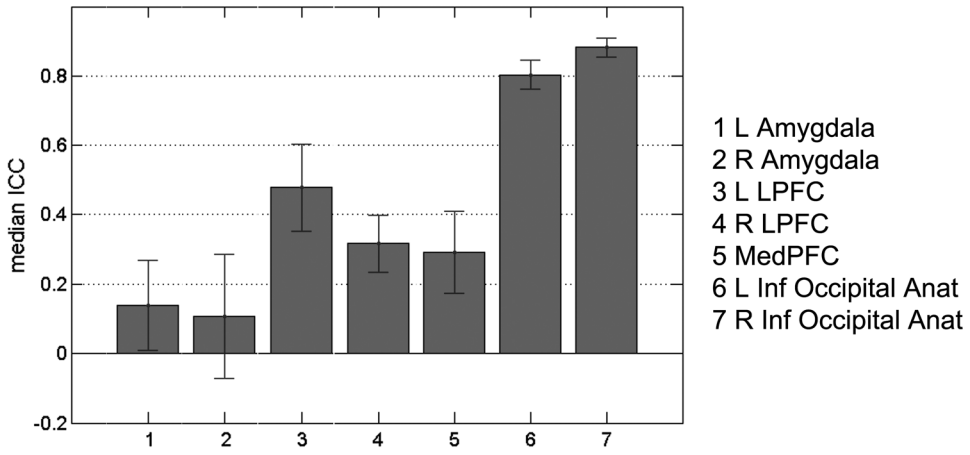

Figure 7. Intraclass Correlation Coefficients (ICC) based on ROIs at TP1 for 27 subjects (FDR corrected, p<.05; 10 contiguous voxels). *The bars represent ICC-values for the ROIs within the contrast all faces > fixation for TP1 and TP2.*

Discussion

The key questions in this study were whether and to what extent activation the amygdala and prefrontal cortex varies over time in adolescents during emotional face processing. Overall the task successfully activated brain regions in the emotional face processing network (e.g. bilateral amygdala, bilateral lateral PFC and visual cortex). Furthermore, on the group level there was no significant variation in activation patterns over time suggesting that activation during an emotional face processing task is relatively stable. However, analyses investigating test-retest reliability on intra-subject level indicated only fair reliability of PFC areas and poor reliability of bilateral amygdala. This indicates that, on the individual level, there is variability in activation patterns for the specific brain areas (i.e. bilateral amygdala, bilateral lateral PFC) over time.

The results showed that the amygdala was activated during the presentation of both happy and fearful faces, with a slightly stronger response to happy faces. These findings are consistent with the hypothesis that the amygdala is not solely a fear processing node but more a general emotion-

processing node8 (Cunningham et al., 2008; Whalen, 1998). The relatively higher response to happy faces than to fearful faces may be specific for mid-adolescence (Somerville et al., 2011), a period during which there is an imbalance between the subcortical driven emotional and frontal-cortical driven control areas of the brain, that may influence the intensity or extent of the amygdala response during emotional face processing (Dahl, 2004). However, this hypothesis should be tested in more detail in future research.

The medial and lateral PFC showed dissociable responses to emotional faces and the different (un)constrained conditions. That is to say, the medial PFC was only more active when viewing happy faces, and lateral PFC was more active during the presentation of all three emotions, although it was relatively more responsive to fearful faces than to happy and neutral faces. Furthermore, both PFC areas showed effects related to the different conditions and context. Thus, in adolescence the amygdala seems to be an area related to emotion processing in general, while PFC shows more specific activation patterns depending on context and type of emotion.

The sensitivity of PFC areas to the different state questions can be explained by participants not having to indicate their subjective feeling in the passive viewing condition. In other words, participants did not have to actively select a choice alternative or regulate their emotion when viewing faces in this context. Only a few studies directly compared active and passive state questions in combination with different emotions in the same design. For example, a study by Monk et al. (2003b) used a similar task and they demonstrated that brain activation patterns in adults seem to depend mostly on attention states, while adolescents were more responsive to the expressed emotion. Furthermore, the authors found that in adolescents, amygdala activation when viewing fearful faces was strongest during passive viewing. This may suggest that the activation pattern in the amygdala is modulated by the context during which adolescents view emotional faces. No such context effect was found in the present study, but future studies should examine the relation between states and emotions in more detail.

Next, we asked the question how stable these patterns were over time. In the behavioral analyses, there were no effects of time suggesting that participant's reaction time patterns and subjective scoring of emotional faces were stable over a period of six months. This finding was confirmed by the ICC analyses on the reaction times and subjective scoring that showed excellent values for almost all conditions. Furthermore, it is interesting to see that the ICC values of the stress-level rating showed an inconsistent pattern: the ICC values for the comparison TP1-TP2 and TP1-TP3 were very low while the comparison TP2-TP3 resulted in an excellent ICC-value. This finding can be explained by the scanner stress that participants probably experience during the first measurement but not during the second and third measurement. The whole brain longitudinal analyses indicated similar recruitment of the face processing network during a period of six months, a finding that was further confirmed by ROI analyses. However, the results of the ICC analyses showed that these findings could not be generalized to individuals. That is to say, ICC-values of the inferior occipital cortex were excellent, but ICC-values for the bilateral lateral PFC and bilateral amygdala were respectively fair and poor indicating large variability in activation patterns over time. These results correspond to the findings of Plichta et al. (2012) who also found low ICC-values for amygdala activation over time during an emotional face-processing task (face matching) in adults (mean scan interval was 14.6 days). Possibly, amygdala activation fluctuates in general in both adults and adolescents. On the individual level, this would not correspond with the results of earlier studies (Monk et al., 2003b) that indicated that amygdala activation is mainly influenced by the context in which adolescents view emotional faces. However, these kinds of studies used group-level analyses, for which we found stable activation. Apparently, when using group-level analyses the within subject variation is cancelled out between subjects which in turn results in stable activation patterns, i.e. when subject one scores high on a variable and subject two scores low, than the average is still in the middle. Contrary, in the ICC analyses the within-subject variation is taken into account leading

to more specific analyses and sometimes less stable results.

Another explanation for the large variability in amygdala activation can be related to habituation effects of amygdala response. However, a study by Johnstone et al. (2005) suggested that the habituation effect only lasts for approximately two weeks and that habituation resets with longer time periods. In their study fifteen adults were scanned three times (0, 2 and 8 weeks) and performed a passive face viewing task. The results of this study indicated that for neutral faces there was a habituation effect after two weeks, i.e. participants showed less activation in the left amygdala. However, this effect was diminished at the 8-week scan session. These findings make it unlikely that habituation effects influenced the current findings, because the test interval of approximately three months is larger compared to prior studies.

Finally, it may be possible that the low test-retest reliability is explained by the fact that we only included healthy participants that did not show a large amount of variability. Future studies should perform comparable analyses including healthy and clinical participants like adolescents with anxiety and/or depression.

There are some limitations in the current study that should be mentioned. First, the subsamples of N=22 and N=18 for the longitudinal analyses were relatively small. Nevertheless, the results we found correspond to the existing literature on face processing in adults and adolescents and also the result of large variability over time in the amygdala is supported by prior literature (Plichta et al., 2012). A second limitation was the relatively broad age-range (12-19 years). Earlier studies suggested that developmental differences in brain activation linked to emotional face processing occur in this developmental phase (Casey et al., 2011; Dahl, 2004; Scherf et al., 2011). In the current study we did not find any age effects, similar to other studies with adolescent groups of a similar age range (Hare et al., 2008; Somerville et al., 2011; Williams et al., 2006). Yet, future studies should replicate these findings and should further investigate the possible influence of puberty on brain activation patterns related to emotional face processing. Finally, this

study included more females than males (24 vs. 3) due to the larger EPISCA study design in which also two clinic groups are included that mainly consist of females. Due to the very small number of boys in the current study it is not expected that the results are influenced by the imbalance in sex. Furthermore, the current sample size of male participants is too small to make any firm conclusions about the influence of sex on the results reported. It would have been very interesting to investigate the influence of menstrual cycle on amygdala activation patterns, due to the large proportion of females included in this study. Previous research by Derntl et al. (2008) indicated that amygdala activation patterns during emotional face processing are influenced by the level of progesterone, which relates to the menstrual phase females are in. Unfortunately, we did not collect the necessary information to perform these analyses. Future studies should investigate this relation in light of our current findings.

Knowledge about variability over time of amygdala and PFC activation in relation to emotional face processing has important implications for clinical conditions, such as anxiety and depression. These conditions are associated with heightened amygdala activation when viewing fearful faces, and especially amygdala activation during emotional face processing is often seen as an important characteristic of anxiety disorders (Mcclure et al., 2007a). A study by (Mcclure et al., 2007a) used between-group level analyses to indicate whether there were differences across measurements. They investigated whether there were fMRI predictors of treatment outcome in a sample of children/adolescents who were predominantly diagnosed with generalized anxiety disorder. The results of their study indicated that participants who responded better to treatment (medication or cognitive behavior therapy) had more left amygdala activation before treatment. These findings are obviously highly relevant as to understand treatment effects at a group level. Future studies should examine whether these patterns are also found for individual analyses.

Taken together, the current study showed that longitudinal analyses

can reveal to what extent neural activation is variable over time in healthy adolescents. Specifically, findings on a group level do not necessarily extend to the individual level. In future research, it will be important to investigate test-retest reliability in clinical samples and to compare these results with the results found in our study. Such studies will set the stage to examine the influence of treatment effects on changes in behavioral and neural levels.

Supplemental material

Supplementary table 1. Whole brain activation patterns for the contrasts: A. all faces > fixation, B. All fearful faces > fixation, C. all happy faces > fixation and D. all neutral faces > fixation. *Regions represent clusters of significant activation at p<.05, FDR-corrected, 10 contiguous voxels, coordinates listed are in MNI space and represent peak values. * = p<.05 when corrected for multiple comparisons at cluster-level*

Contrast	Region	Side	z-score	x	y	z	
A.							
All faces > Fixation	Fusiform gyrus	R	7.10	33	-78	-12	*
	Inferior frontal gyrus	L	5.31	-48	42	-12	*
	Inferior frontal gyrus	L	3.09	-36	24	-15	
	Middle frontal gyrus	R	4.11	48	48	9	*
	Superior frontal gyrus	L	3.93	-3	24	48	
	Postcentral gyrus	R	3.53	60	-18	24	
	Inferior temporal gyrus	R	4.53	30	-6	-42	*
	Parahippocampal gyrus	R	3.56	21	-33	-3	
	Amygdala	L	3.24	-30	-3	-24	
	Caudate body	L	3.42	-12	-3	24	
	Caudate tail	L	3.33	-24	-36	18	
	Insula	L	3.41	-39	-3	15	
	Insula	R	3.05	39	-3	6	
B.							
Fearful faces > Fixation	Fusiform gyrus	R	7.40	33	-78	-12	*
	Inferior frontal gyrus	L	5.75	-48	42	-12	*
	Inferior frontal gyrus	L	3.06	-56	18	3	
	Inferior frontal gyrus	L	3.84	-36	21	-15	
	Inferior frontal gyrus	R	4.20	48	48	6	*
	Superior frontal gyrus	L	4.10	-3	24	48	
	Precentral gyrus	R	3.46	54	-3	48	
	Postcentral gyrus	R	3.25	63	-18	27	
	Hippocampus	L	3.16	-30	-33	-3	
	Parahippocampal gyrus	R	3.07	18	-33	-3	
	Uncus	L	3.62	-30	-3	-27	
	Uncus	R	4.27	30	-3	-42	*
C.							
Happy faces > Fixation	Fusiform gyrus	R	6.90	36	-78	-12	*
	Inferior frontal gyrus	L	4.15	-48	42	-15	
	Inferior frontal gyrus	L	3.79	-48	39	9	
	Middle frontal gyrus	R	4.21	39	30	12	
	Postcentral gyrus	R	3.15	63	-21	30	
	Inferior temporal gyrus	R	3.99	30	-6	-42	
	Parahippocampal gyrus	R	3.63	21	-33	-3	
	Amygdala	L	3.43	-27	-3	-24	
	Amygdala	R	4.53	30	-3	-21	
	Caudate body	L	3.00	-12	-3	27	
	Caudate tail	L	3.34	-24	-36	15	
	Insula	L	3.42	-45	-3	9	
	Insula	R	3.42	39	-3	6	
D.							
Neutral faces > Fixation	Fusiform gyrus	R	6.75	39	-75	-15	*
	Inferior frontal gyrus	L	4.34	-48	42	-15	
	Inferior frontal gyrus	R	3.67	51	45	3	
	Superior frontal gyrus	L	3.62	-3	24	48	
	Postcentral gyrus	R	3.54	60	-18	21	
	Inferior temporal gyrus	R	3.91	30	-6	-42	
	Parahippocampal gyrus	R	3.78	24	-33	0	
	Amygdala	R	3.44	27	-6	-15	
	Caudate (body)	L	3.72	-9	0	24	
	Caudate tail	L	3.89	-24	-36	18	
	Insula	R	3.07	42	-3	12	

Supplementary table 2. Whole brain activation patterns for the contrasts: A. all faces > fixation, B. All fearful faces > fixation, C. all happy faces > fixation and D. all neutral faces > fixation. *Regions represent clusters of significant activation at p<.05, FDR-corrected, 10 contiguous voxels, coordinates listed are in MNI space and represent peak values. * = p<.05 when corrected for multiple comparisons at cluster-level*

Contrast	Region	Side	z-score	x	y	z	
A.							
All faces > Fixation	Fusiform gyrus	R	Inf.	36	-78	-15	*
	Inferior frontal gyrus	L	4.31	-48	39	3	
	Inferior frontal gyrus	R	3.86	51	42	12	
	Superior frontal gyrus	L	3.68	-3	24	48	
	Amygdala	L	3.20	-18	-6	-21	
	Uncus	R	4.48	21	-3	-24	
	Uncus	R	3.35	30	-3	-39	
B.							
Fearful faces > Fixation	Fusiform gyrus	R	Inf.	36	-78	-15	*
	Inferior frontal gyrus	L	4.69	-48	39	3	
	Inferior frontal gyrus	L	3.03	-36	21	-15	
	Middle frontal gyrus	4	3.70	51	39	24	
	Superior frontal gyrus	L	3.41	-3	24	48	
	Uncus	R	4.08	21	-3	-24	
C.							
Happy faces > Fixation	Fusiform gyrus	R	Inf.	36	-78	-12	*
	Inferior frontal gyrus	L	4.31	-48	39	6	
	Inferior frontal gyrus	L	3.55	-27	24	-21	
	Inferior frontal gyrus	L	3.63	-48	42	-12	
	Inferior frontal gyrus	R	4.47	51	39	12	
	Superior frontal gyrus	L	3.22	-3	24	48	
	Amygdala	L	3.82	-18	-6	-21	
	Uncus	R	3.22	30	-3	-42	
	Uncus	R	5.47	21	-3	-24	
D.							
Neutral faces > Fixation	Fusiform gyrus	R	Inf.	36	-78	-15	*
	Inferior frontal gyrus	L	3.12	-51	39	-9	
	Inferior frontal gyrus	R	3.24	51	39	12	
	Middle frontal gyrus	R	3.36	33	-3	-42	
	Superior frontal gyrus	L	3.21	-3	24	48	
	Uncus	R	3.39	21	-3	-24	

Supplementary table 3. A. shows the ICC-values per ROI within the contrast all fearful faces > fixation, B. shows the ICC-values per ROI within the contrast all happy faces > fixation and C. shows the ICC-values per ROI within the contrast all neutral faces > fixation.

ROIs are based in the full sample of N=27 subject at TP1 (FDR corrected, p<.05; 10 contiguous voxels). Interpretation of ICC-values: poor (<0.4), fair (0.41–0.59), good (0.60–0.74) or excellent (>0.75). Abbreviations: Fix, Fixation; TP, Time point; medICC, Median intraclass correlation coefficient; SE, Standard error; ROI; Region of interest; L, left; Anat, Anatomical ROI derived from Marsbar AAL regions; R; Right; LPFC; lateral prefrontal cortex; InfTri; inferior triangularis; MedPFC, medial prefrontal cortex; Inf, Inferior.

A.

Fearful faces > Fixation	TP1-TP2 N22	TP1-TP2 N18	TP2-TP3 N18	TP1-TP3 N18
ROI	medICC (SE)	medICC (SE)	medICC (SE)	medICC (SE)
L Amygdala Anat	0,14 (0,12)	0,15 (0,16)	0,30 (0,11)	0,30 (0,07)
L Amygdala	0,17 (0,10)	0,21 (0,09)	0,20 (0,13)	-0,12 (0,11)
L Amygydala Masked	0,24 (0,08)	0,27 (0,10)	0,15 (0,23)	0,06 (0,18)
R Amygdala Anat	0,21 (0,08)	0,24 (0,08)	0,24 (0,08)	0,33 (0,10)
R Amygdala	0,14 (0,20)	0,18 (0,22)	0,12 (0,14)	0,14 (0,09)
R Amygydala Masked	0,13 (0,11)	0,16 (0,12)	0,17 (0,13)	0,34 (0,10)
L LPFC	0,37 (0,13)	0,37 (0,13)	0,33 (0,14)	0,36 (0,12)
L LPFC InfTri Masked	0,38 (0,14)	0,32 (0,12)	0,44 (0,12)	0,49 (0,09)
R LPFC	0,34 (0,06)	0,34 (0,05)	0,32 (0,10)	0,28 (0,05)
R LPFC InfTri Masked	0,32 (0,10)	0,32 (0,10)	0,34 (0,08)	0,27 (0,07)
MedPFC	0,23 (0,11)	0,28 (0,09)	0,07 (0,09)	0,54 (0,11)
L Occipital Inf	0,85 (0,04)	0,83 (0,06)	0,85 (0,05)	0,83 (0,05)
R Occipital Inf	0,89 (0,02)	0,88 (0,03)	0,86 (0,05)	0,89 (0,03)

B.

Happy faces > Fixation	TP1-TP2 N22	TP1-TP2 N18	TP2-TP3 N18	TP1-TP3 N18
	medICC (SE)	medICC (SE)	medICC (SE)	medICC (SE)
L Amygdala Anat	-0,02 (0,12)	-0,02 (0,12)	0,19 (0,10)	0,15 (0,07)
L Amygdala	0,03 (0,07)	0,02 (0,07)	0,15 (0,14)	0,14 (0,06)
L Amygydala Masked	-0,02 (0,14)	0,01 (0,14)	-0,03 (0,22)	0,11 (0,10)
R Amygdala Anat	0,04 (0,11)	0,01 (0,11)	0,25 (0,09)	0,37 (0,11)
R Amygdala	0,04 (0,15)	0,04 (0,17)	0,22 (0,06)	0,16 (0,06)
R Amygydala Masked	0,09 (0,15)	0,06 (0,17)	0,33 (0,12)	0,43 (0,10)
L LPFC	0,43 (0,13)	0,43 (0,16)	0,57 (0,12)	0,40 (0,14)
L LPFC InfTri Masked	0,40 (0,12)	0,41 (0,15)	0,61 (0,08)	0,45 (0,11)
R LPFC	0,31 (0,08)	0,29 (0,09)	0,31 (0,13)	0,23 (0,09)
R LPFC InfTri Masked	0,32 (0,08)	0,31 (0,09)	0,24 (0,10)	0,28 (0,13)
MedPFC	0,23 (0,10)	0,27 (0,09)	0,19 (0,13)	0,43 (0,19)
L Occipital Inf	0,81 (0,05)	0,78 (0,08)	0,82 (0,06)	0,81 (0,05)
R Occipital Inf	0,86 (0,04)	0,85 (0,05)	0,89 (0,03)	0,89 (0,03)

C.

Neutral faces > Fixation	TP1-TP2 N22	TP1-TP2 N18	TP2-TP3 N18	TP1-TP3 N18
	medICC (SE)	medICC (SE)	medICC (SE)	medICC (SE)
L Amygdala Anat	0,07 (0,14)	0,09 (0,16)	0,28 (0,06)	0,24 (0,08)
L Amygdala	0,08 (0,09)	0,11 (0,08)	-0,06 (0,11)	0,13 (0,09)
L Amygydala Masked	0,05 (0,20)	0,09 (0,21)	0,01 (0,14)	0,09 (0,12)
R Amygdala Anat	0,15 (0,12)	0,23 (0,11)	0,30 (0,10)	0,19 (0,13)
R Amygdala	0,01 (0,09)	0,08 (0,09)	0,19 (0,05)	0,17 (0,09)
R Amygydala Masked	0,09 (0,14)	0,18 (0,15)	0,31 (0,07)	0,16 (0,12)
L LPFC	0,44 (0,11)	0,43 (0,11)	0,33 (0,10)	0,18 (0,15)
L LPFC InfTri Masked	0,26 (0,12)	0,26 (0,12)	0,39 (0,20)	0,16 (0,16)
R LPFC	0,31 (0,08)	0,30 (0,08)	0,28 (0,14)	0,21 (0,10)
R LPFC InfTri Masked	0,28 (0,10)	0,27 (0,11)	0,38 (0,12)	0,31 (0,10)
MedPFC	0,23 (0,13)	0,28 (0,12)	0,16 (0,07)	0,24 (0,15)
L Occipital Inf	0,78 (0,05)	0,74 (0,04)	0,83 (0,06)	0,79 (0,04)
R Occipital Inf	0,87 (0,03)	0,86 (0,04)	0,87 (0,04)	0,86 (0,04)

Chapter 5

Longitudinal changes
in amygdala activation

Submitted for publication as:
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.

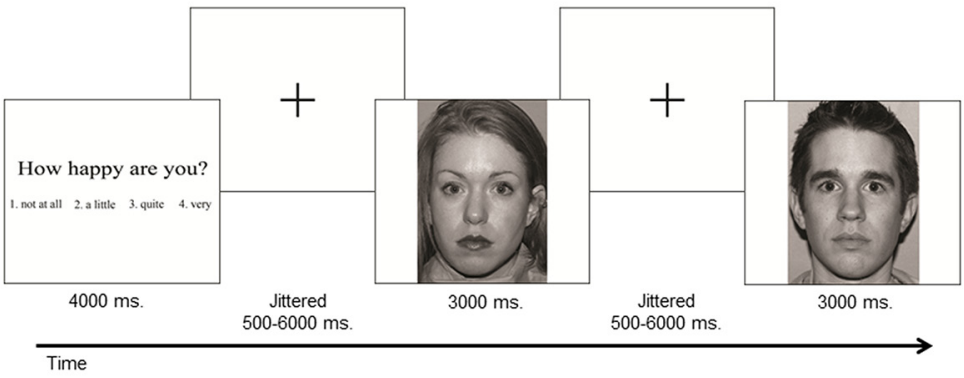

Figure 1. Emotional face-processing task. *Participants were presented with one of the state questions (i.e., how happy are you, how afraid are you, how wide is the nose or passive viewing) followed by a fixation cross. Thereafter, one picture of a negative, positive or neutral face was shown during 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.

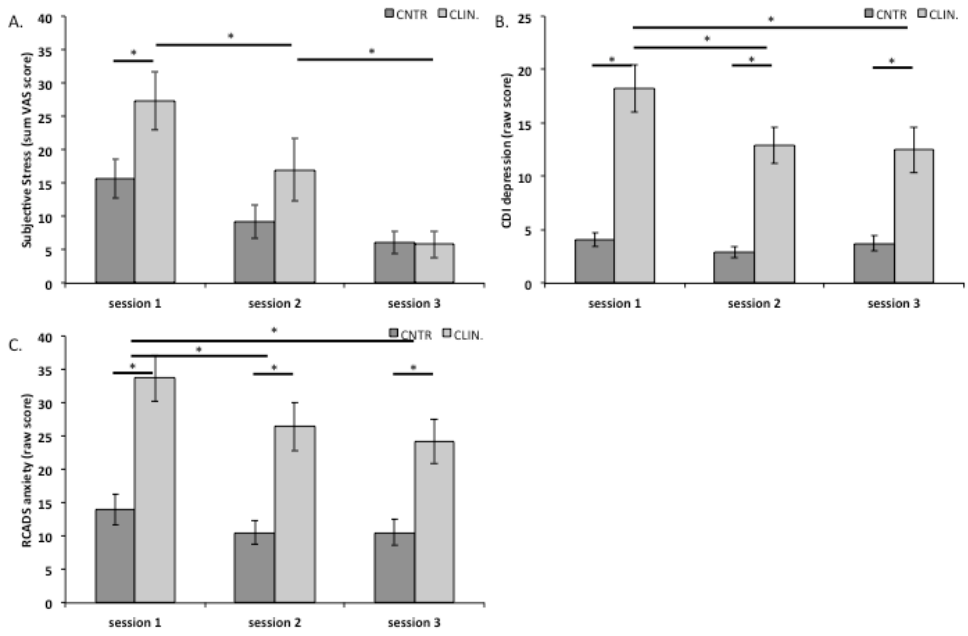

Figure 2. Subjective stress rated on a Visual Analogue Scale (VAS) during scanning (A), symptoms of depression measured with the Children's Depression Inventory (CDI) (B) and symptoms of anxiety over time measured with the anxiety subscale of the Revised Children's Anxiety and Depression Scale (RCADS) (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.

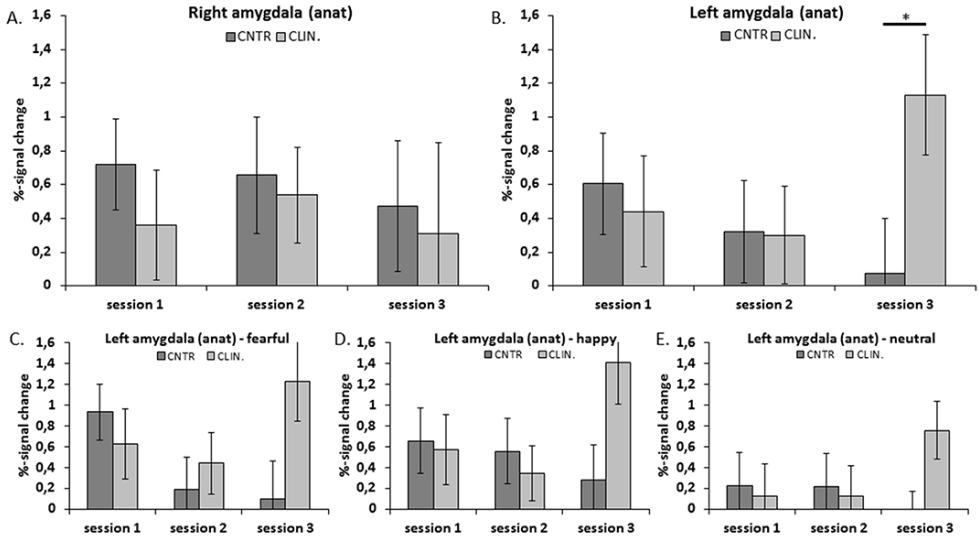

Figure 3. Time related changes in amygdala reactivity in the clinical group and the control group. *(A) Interaction between session and group for left and right amygdala, (B) Interaction between session and group for left amygdala separately for fearful, happy and neutral faces and collapsed across state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

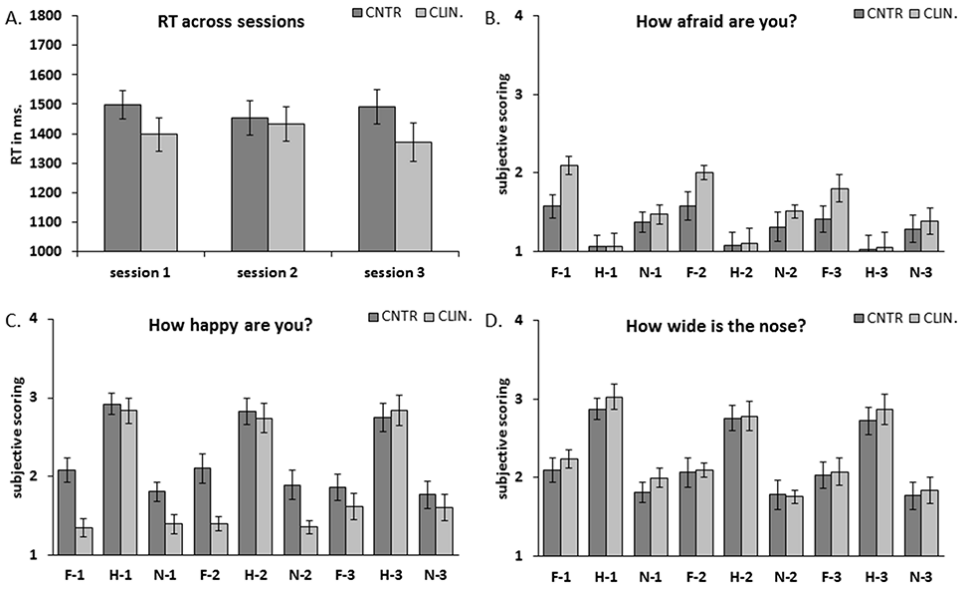

Figure S1. Behavioral scores for the emotional face-processing task. *(A) Mean reaction times in milliseconds collapsed across emotions and state questions. (B, C, D) represent mean subjective scoring per state question for both groups during all three sessions. CNTR=control group; CLIN.=clinical group;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

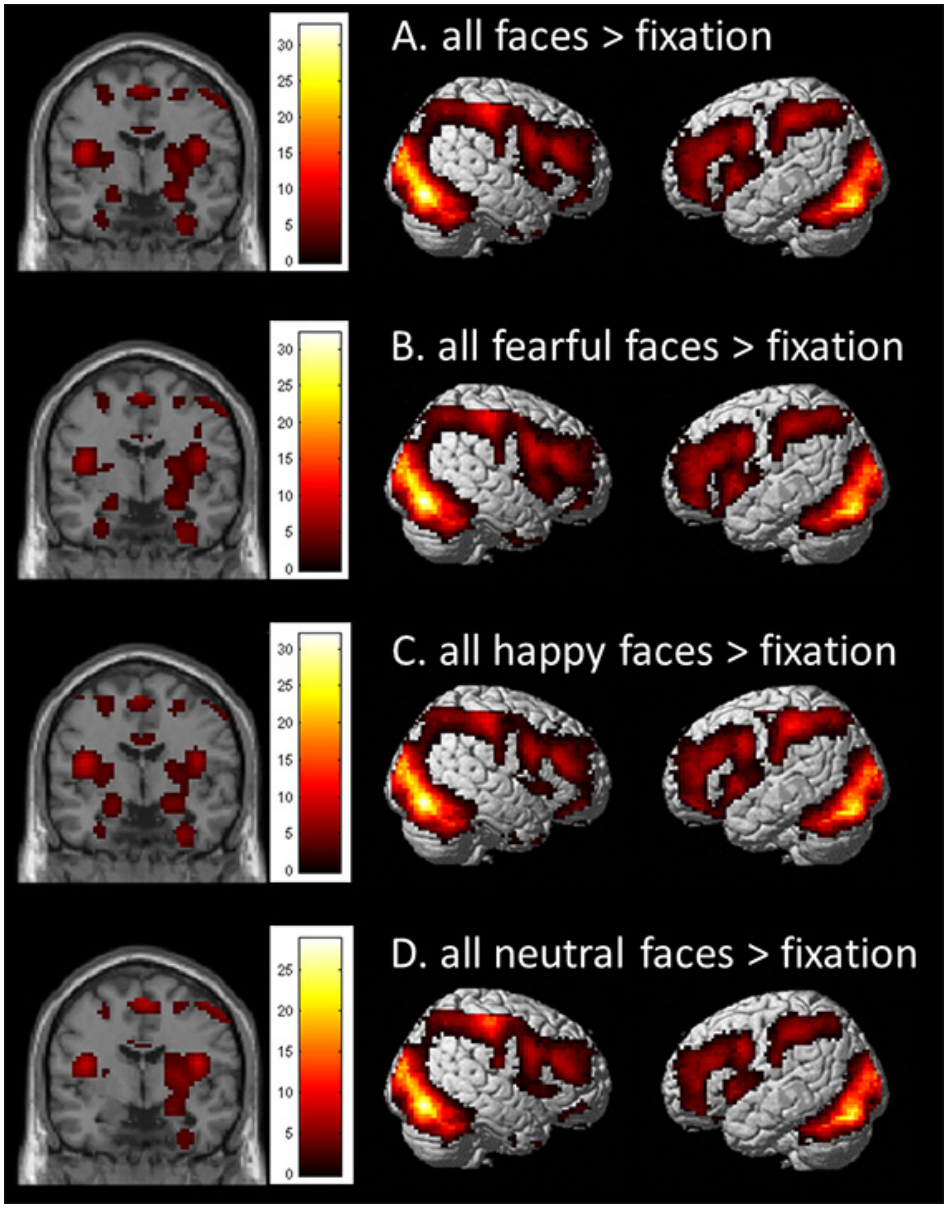

Figure S2. Whole brain activation patterns within the complete sample of N=42 adolescents per session for the contrasts: A. all faces > fixation, B. all fearful faces > fixation, C. all happy faces > fixation, and D. all neutral faces > fixation. *Coordinates represent significant peaks of activation 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	

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).

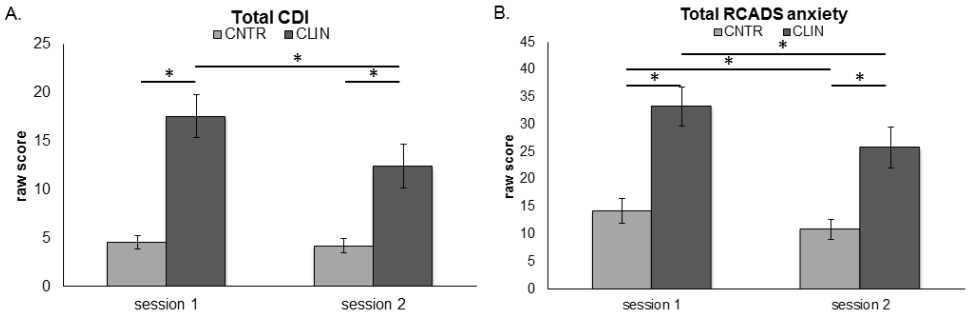

Figure 1. Overview of longitudinal changes in (a) self-reported depression symptoms (CDI) and (b) self-reported anxiety symptoms (RCADS). ** p < 0.05; CNTR = control group; CLIN. = clinical group.*

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.

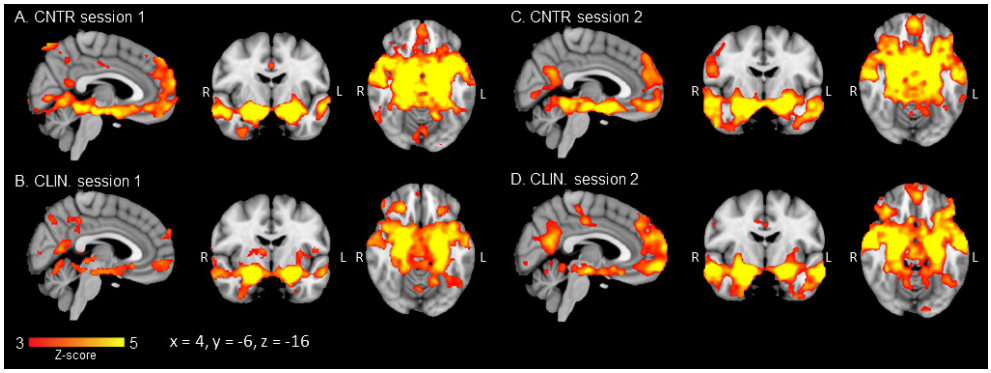

Figure 2. Positive bilateral amygdala connectivity for (a) the control group at session 1, (b) the clinical group at session 1, (c) the control group at session 2 and (d) the clinical group at session 2. *Bilateral amygdala shows positive connectivity with several subcortical and cortical regions. Images 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).

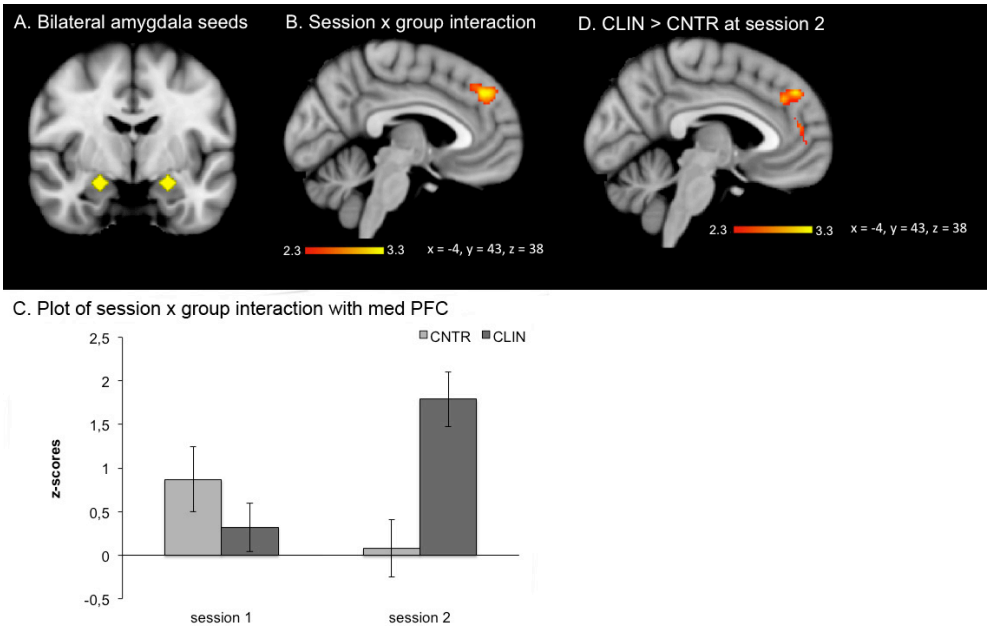

Figure 3. Overview of time related changes in functional connectivity from the amygdala: *(a) bilateral amygdala seeds (central voxel: ±22, -6, -16; 4 mm sphere), (b) significant positive connectivity between right amygdala and medial PFC as revealed by a session x group interaction, (c) z-values for 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

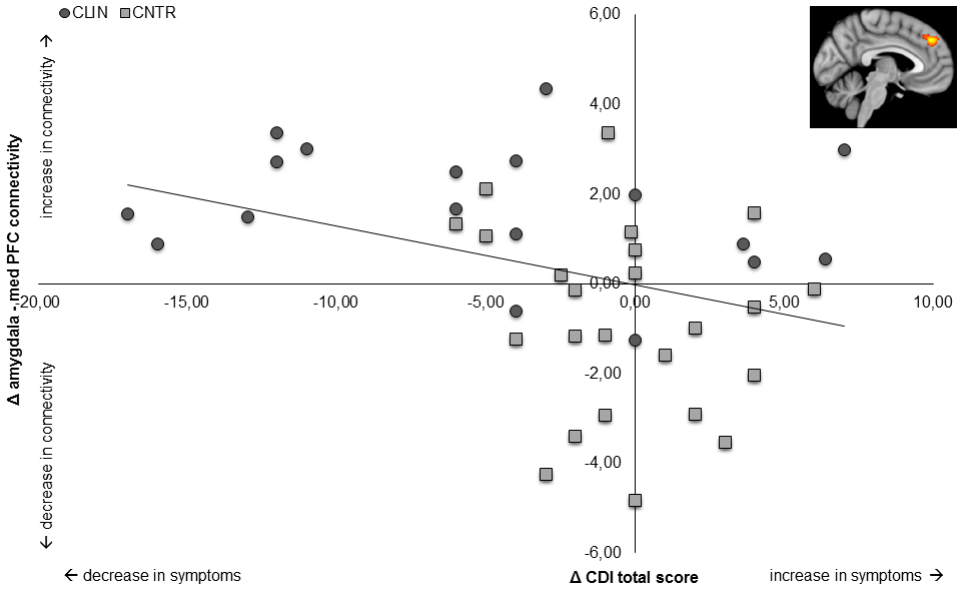

Figure 4. Negative correlation between change in right amygdala – medial PFC connectivity and change in self-reported depression symptoms. *Positive values on x-axis indicate an increase in depression symptoms over time while negative values indicate a decrease in depression symptoms. For the y-axis,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

Chapter 7

Summary and discussion

Summary

The goal of this thesis was to examine the neurobiological mechanisms of depression and anxiety using a specific focus on amygdala activity and connectivity. There were three main objectives: (1) to examine whether adolescents with depressive and anxiety disorders showed differentiating patterns of amygdala activation during an emotional face processing task, (2) to investigate the test re-test reliability of the fMRI signal in several brain regions related to emotional face processing and (3) to study longitudinal changes in amygdala activity and connectivity in a sample of depressed and anxious adolescents who were referred for cognitive behavioral therapy based treatment. To answer the questions related to these goals and objectives, we conducted a large longitudinal fMRI study to examine the neurobiological mechanisms of depression and anxiety and childhood sexual abuse, called EPISCA (Emotional Pathways' Imaging Study in Clinical Adolescents). This thesis focused on the adolescents with a depressive or anxiety disorder and a sample of normally developing adolescents was used as a control group. In the following sections, the main findings and conclusions are presented. The chapter ends with limitations and recommendations for future studies.

Amygdala reactivity in response to emotional faces

In ***chapter 2***, a study was described investigating amygdala reactivity in response to emotional faces in a sample of adolescents with a DSM-IV depression or anxiety diagnosis and a healthy control group. It was hypothesized that depressed and anxious adolescents would show higher levels of amygdala activity in response to fearful faces compared to healthy adolescents. Furthermore, it was hypothesized that there would be a positive correlation between levels of self-reported depression and anxiety symptoms and amygdala reactivity. The results showed strong activation in brain regions previously related to emotion processing like the dorsolateral prefrontal cortex, the amygdala and the visual cortex (Costafreda et al., 2008; Fusar-Poli et al., 2009). Whole brain comparisons did not reveal significant diffe-

rences in amygdala activation between de depression/anxiety group and the healthy control group. Follow-up region of interest (ROI) analyses for left and right amygdala only resulted in significant effects for the emotion presented: amygdala reactivity was higher for fearful and happy faces than for neutral faces. Again there were no significant group differences. There was however a strong positive correlation between levels of self-reported anxiety symptoms and amygdala reactivity in response to emotional faces (fearful, happy and neutral) within the depression/anxiety group. This corresponds with previous studies, which also reported a positive relation between levels of self-reported anxiety and amygdala activation during an emotional face-processing task (Ball et al., 2012; Monk et al., 2003a; Stein et al., 2007; Thomas et al., 2001a).

Although there was no overall group difference in amygdala activation, the positive correlation between anxiety symptoms and amygdala activation suggest that anxiety symptoms may be an underlying trait characteristic for both depression and anxiety disorders.

Next, we examined patterns of habituation in adolescents with depressive and anxiety disorders, adolescents who experienced childhood sexual abuse (CSA) and healthy control group adolescents ***(chapter 3)***. Previous research indicated that both adolescents with depressive and anxiety disorders and adolescents who experienced CSA show differentiating patterns of amygdala activation (Garrett et al., 2012; Monk et al., 2008b; Roberson-Nay et al., 2006). It was previously found that depressed and anxious adolescents show slower rates of habituation of amygdala activation in response to emotional faces compared to healthy controls (Hare et al., 2008). We hypothesized to find different rates of amygdala habituation in response to emotional faces for the two clinical groups when compared with the control group. Furthermore, we were interested to see whether there were differences between the depressed/anxious group and the group of adolescents who experienced CSA. These two clinical groups show a large overlap in symptomatology, but also have unique characteristics in that the adolescents in the CSA group experienced a traumatic event.

The results of this study showed habituation of amygdala activation in the healthy control group and differentiating patterns of amygdala habituation in the two clinical groups: depressed and anxious adolescents showed comparable levels of amygdala activation as the control group but they did not show a significant decline in activation, while the adolescents who experienced CSA showed an initial increase in activation in the amygdala followed by a relatively fast habituation to a level comparable to that of the two other groups. These results were found on whole brain and ROI level indicating robust findings. Although speculative, the increased amygdala activity in the CSA group may be related to an increased vigilance to emotional faces that is caused by the experience of a traumatic event. However, the down-regulation of this primary emotional response in the CSA group might be intact, which can result in relatively fast habituation over runs. In adolescents with depressive and anxiety disorders the primary emotional response (increase in amygdala activation) seems to be less exaggerated, although the integration of information by cognitive control regions may be insufficient and cause emotion regulation problems. This hypothesis would fit with the suggested top-down regulation model for depression in which it is stated that depressive symptoms originate from an inefficient top-down regulation by the prefrontal cortex (Mayberg, 1997).

Reliability of fMRI signal

A new direction to examine individual changes in neurobiological mechanisms is by performing longitudinal studies. When using these repeated measure designs it is important to test whether patterns of brain activation vary over time in healthy individuals. There is some research investigating the test re-test reliability of the fMRI signal in adult participants (Johnstone et al., 2005; Plichta et al., 2012), while comparable research in adolescents is missing even though adolescence is a period in life during which significant changes in emotional functioning occur (Dahl, 2004).

In ***chapter 4*** we described a study investigating the test re-test reli-

ability of the fMRI signal in several brain regions related to emotional face processing. We included a sample of healthy adolescents that were scanned three times in a six-month period. Whole brain results indicated activation in regions related to emotional face processing: bilateral amygdala, bilateral dorsolateral prefrontal cortex and visual cortex. Furthermore, behavioral, whole brain and ROI analyses showed no significant effects of time suggesting stable patterns of activation over time. However, the results of the test re-test reliability analyses showed that there was substantial within-subject variability in dorsolateral prefrontal cortex and amygdala activation: Test re-test reliability values for occipital cortex were high, for dorsolateral prefrontal cortex reasonable and for amygdala low. These findings suggest substantial within-subject variance for amygdala activation during an emotional face processing task, which is not visible in group based analyses as used in whole brain or ROI analyses. In these analyses, the within subject variance might be cancelled out by averaging the activation. These findings correspond to results reported in studies including adult participants (Plichta et al., 2012) and provide us with important information about stability of the fMRI signal in several brain regions. Longitudinal studies should take these findings into account when interpreting their results.

Longitudinal changes in amygdala functioning

Prior research indicated that there are pronounced differences in amygdala activity between adolescents with depressive and anxiety disorders and healthy control group adolescents (Monk et al., 2008a; Monk et al., 2008b; Perlman et al., 2012; Thomas et al., 2001a). However, these studies often used only one measurement and they did not test the longitudinal changes of the underlying neurobiological mechanisms of depression and anxiety. There are some studies that followed participants with a depression or anxiety disorder over time, but these only included one fMRI session at the start the study (Canli et al., 2005; Siegle et al., 2006). The results of these studies suggest that over the course of treatment the reactivity of the amyg-

dala decreases. Possibly these results relate to the increase of top-down regulation of the prefrontal cortex reported in other studies (Clark, & Beck, 2010; Quide et al., 2012). These studies however often included adult participants. Only one other study examined longitudinal changes in amygdala activation in adolescents with an anxiety disorder. They reported an increase in amygdala activation over time in a sample of anxious adolescents referred for cognitive behavioral therapy (Maslowsky et al., 2010).

To further investigate the longitudinal changes in amygdala activation in depressed and anxious adolescents, we conducted a longitudinal fMRI study in which depressed and anxious adolescents and healthy control group adolescents were scanned three times over a six-month period ***(chapter 5)***. During each scan session the participants performed an emotional face processing task including fearful, happy and neutral faces. The results of this study showed a significant decrease in self-reported depression and anxiety symptoms in the clinical group. Furthermore, there was a significant interaction between group and session in the left amygdala: at session one and session two there were no significant differences between the clinical group and the control group but at session three the clinical group showed significantly more amygdala activity in response to processing emotional faces compared to the control group. This effect was independent of the emotional face depicted. Overall, these results are in line with the finding reported by Maslowsky and colleagues (2010), because they also showed an increase in amygdala activation in a sample of adolescents diagnosed with generalized anxiety disorder who were referred for CBT-based treatment. Furthermore, recent studies suggested that adolescents show a prolonged process of fear extinction after fear conditioning, which might result in increased sensitization of amygdala reactivity to emotional stimuli. (Drysdale et al., 2013; Pattwell et al., 2012). Further research is necessary to examine the robustness of these effects and to see whether they relate to changes in symptomatology and/or treatment outcome.

Besides examining longitudinal changes in amygdala activity in res-

ponse to emotional faces, we also examined longitudinal changes in resting state functional connectivity (RSFC). Previous research has indicated that there are group differences between adolescents with depressive and anxiety disorders and healthy controls in functional connectivity between the amygdala and several regions, including medial prefrontal cortex (Hulvershorn et al., 2011; Pannekoek et al., 2014a). However, not much is known about longitudinal changes in RSFC within the limbic network. In ***chapter 6***, a study was described investigating longitudinal changes in RSFC in adolescents with depressive and anxiety disorders and healthy control group adolescents. RSFC data was collected at two occasions that were separated from each other by a six-month period during which the depressed and anxious adolescents received treatment as usual (cognitive behavioral therapy based). A seed-based region of interest approach was used with seeds in the bilateral amygdala. The results showed a significant interaction between group and session in which the depressed and anxious adolescents showed a large increase in positive connectivity between the right amygdala and medial prefrontal cortex (PFC). In addition, a significant negative correlation was found between change in right amygdala – medial PFC connectivity and change in self-reported depression symptoms within the complete sample. Adolescents who showed a larger increase in positive connectivity also showed a larger decrease in depression symptoms. Although causality cannot be derived from RSFC analyses, possibly these results indicate an increase in top-down regulation by the medial PFC, which corresponds to a proposed model on depression (Mayberg, 1997). This model suggests that depressive symptoms are caused by ineffective top-down regulation by the prefrontal cortex over the primary emotional response of the amygdala. Other studies that investigated connectivity in depressed and anxious adults supported these models (Clark, & Beck, 2010; Månsson et al., 2013). Future research should further investigate these effects by examining adolescents with depressive and anxiety disorders who are referred for different forms of treatment, such as structured cognitive behavioral therapy procedures or medication.

Conclusions

The studies in this thesis aimed to further investigate the neurobiological mechanisms of adolescent onset depression and anxiety disorders by using a longitudinal study design that included both task related brain activation and resting state functional connectivity (RSFC). It was demonstrated that adolescents with depressive and anxiety disorders show differentiating patterns of amygdala reactivity and connectivity compared to a healthy control group. The findings indicate that the amygdala indeed is an important region involved in emotional face processing and that focusing on this region can provide further insights in the development and persistence of depressive and anxiety disorders in adolescents. Furthermore, using a dimensional approach and taking individual differences in self-reported depression and anxiety symptoms into account highlighted the role of self-reported anxiety symptoms in amygdala reactivity during emotional faces processing. In the following sections I will provide some general consideration and directions for future research.

Group comparisons

In ***chapters 2 and 3*** we described the results of two studies using group comparisons and data of only one session. We reported a strong positive relation between self-reported anxiety symptoms and amygdala activation, a relation that was not present for self-reported depression symptoms. Furthermore, there were group differences in habituation rate: depressed and anxious adolescents showed no habituation of amygdala activity to emotional faces while control group adolescents and adolescents who experienced CSA did show habituation.

Since there was only a relation between self-reported anxiety symptoms and amygdala activation and not with self-reported depression symptoms, this might indicate that the level of anxiety symptoms, and not the level of depression symptoms, is an important predictor for differentiating patterns of amygdala activation in these clinical groups. Not all studies that

examined amygdala activation in depressed and anxious adolescents included self-report questionnaires about symptomatology. When reviewing the existing literature on amygdala activation and face processing in depressed and anxious adolescents, there are more indices that highlight the importance of anxiety symptoms for differentiating amygdala activity: the studies including anxious adolescents report more consistent findings of increased amygdala activity (Mcclure et al., 2007b; Monk et al., 2008b), while the studies including depressed adolescents are more often inconsistent (Monk et al., 2008a; Roberson-Nay et al., 2006; Thomas et al., 2001a). Therefore, the relation between amygdala activation during emotional face processing and anxiety symptomatology should be studied in more detail.

The lack of habituation in the depressed and anxious adolescents and the increased amygdala activation and fast habituation in the adolescents who experienced CSA are interesting findings that provide new insights into the underlying mechanisms of depression and anxiety. Although speculative, it might be that the onset of adolescent depression and anxiety is predisposed by personality styles like neuroticism. This predisposition might increase the vulnerability for developing depressive and anxiety disorders. In contrast, CSA is by definition the result of a traumatic event. This experience might make CSA adolescents more vigilant to emotional faces, which is expressed by heightened patterns of amygdala activity (Garrett et al., 2012; Hart, & Rubia, 2012). For depression and anxiety this mechanism might work differently: for these adolescents the primary response might be comparable to healthy adolescents while there are differences in top-down regulation by prefrontal regions that can dampen/exaggerate depressive and anxiety symptoms. More research should be performed to further investigate these mechanisms for example by using task designs in which participants have to regulate their emotions.

Longitudinal changes

Chapters 5 and 6 described longitudinal studies in which we investi-

gated longitudinal changes in amygdala activity and connectivity. For both amygdala activity in response to emotional faces and amygdala connectivity we found significant changes over a six-month period. Within the group of adolescents with depressive and anxiety disorders we reported a significant increase in amygdala activity during an emotion face processing task and a significant increase positive amygdala – DMPFC connectivity. The results of the longitudinal task analyses were interpreted as an increased sensitivity of amygdala activation over time that is possibly caused by treatment effects. One previous study reported comparable results namely an increase in amygdala activation over time in a sample of anxious adolescents who were referred for CBT therapy (Maslowsky et al., 2010). Some recent studies provided a possible cause for these effects: adolescents show a prolonged process of fear extinction, which may result in increased sensitization of amygdala reactivity to emotional faces (Drysdale et al., 2013; Pattwell et al., 2012). The results of the longitudinal connectivity analyses showed an increase in positive connectivity between amygdala and DMPFC, which is interpreted as an increase in top-down control by the medial PFC. This interpretation corresponds with the existing literature in adults (Fu et al., 2008; Månsson et al., 2013) and current ideas about the underlying neurobiological mechanisms of depression and anxiety (Mayberg, 1997; Quide et al., 2012).

When combining the results of these two studies, the results can also be interpreted as being complementary to each other: the increase in positive connectivity can be driven by an increase of the primary response in the amygdala. This in turn might lead to increased top-down regulation by the medial prefrontal cortex. The RSFC analyses described in this thesis do not provide information about the directionality of the increase in positive connectivity. Therefore, more sophisticated analyses like dynamic causal modelling are necessary (Friston, Harrison, & Penny, 2003). It would be interesting to combine task-based and RSFC data of large longitudinal samples, to further examine whether depressed and anxious adolescents show an increase in amygdala activity, an increase in top-down regulation by medial

prefrontal cortex or a combination of these mechanisms. Furthermore, such longitudinal studies can examine the influence of changes in self-reported symptomatology and treatment effectivity on changes in brain activity/connectivity.

In ***chapter 4*** we described a longitudinal study investigating test re-test reliability of amygdala, prefrontal cortex and occipital cortex activation during an emotional face processing task. The results indicated that occipital cortex activation is quite stable over time. However, prefrontal cortex activation and especially amygdala activation show much more variation within subjects across test sessions. These results perfectly match previous research that also showed intermediate test re-test reliability for prefrontal regions and low test re-test reliability for the amygdala (Hare et al., 2008; Plichta et al., 2012). Researchers should take the within-subject variability into account when interpreting the results of longitudinal studies, especially when there are more than three measurements. Thus far, fMRI data analyses programs like SPM (Statistical Parametric Mapping; Welcome Department of Cognitive Neurology, London) have not yet implemented many flexible statistical models, like flexible factorial or multi-level models that are appropriate for analyzing longitudinal data on whole brain level. One statistical model that is implemented on SPM is the flexible factorial model. Within this model you can indicate that one subject is tested multiple times and that the within-subject variability should be taken into account. However, not all available methods for whole brain analyses are as flexible as necessary. Especially when more than three measurements are included, more complex covariance matrices should be applied to model the within-subject variability correctly. For Region of Interest (ROI) analyses better mathematical approaches are available like repeated measurement ANOVA or multi-level analyses. Future research, hopefully leads to the implementation of more complex statistical models to perform longitudinal analyses on whole brain level.

Limitations and future directions

It is important that other research groups replicate the findings reported in this thesis. When doing this, the following suggestions should be taken into account. First of all, a longitudinal design should be used in which there is a pre-treatment measurement, a post-treatment measurement and a follow-up measurement. By using such a design it is possible to make firmer conclusions about the influence of treatment on changes in amygdala activation or connectivity. Also, it provides us with the opportunity to evaluate which adolescents benefit from treatment and which ones do not. In the current study design we intended to include pre-, post- and follow-up measurements, however, since we used treatment as usual some adolescents already finished their therapy by the second measurement while others were still receiving treatment when coming in for the third measurement.

Related to this, studies should apply standardized forms of treatment. One example of this is a structured CBT protocol in which all adolescents receive an equal amount of treatment sessions with the same content. It would also be important and interesting to compare two sorts of therapy within the same design: for example a medication group can be added. Research has indicated that both CBT and medication can be effective for treating depression and anxiety and that a combination of both is even more effective (Compton et al., 2004; Walkup et al., 2008). However, it is not clear to what extent these different forms of treatment influence the underlying neurobiological mechanisms of depression and anxiety. The studies described in this thesis included unstructured CBT-based treatment. Because of the collaboration between different institutes, we were not able to apply the same structures therapy to all participants. For this reason, our conclusions are based on time related changes and the relation with changes in self-reported symptomatology instead of treatment related changes.

Finally, future research should include larger samples of depressed and anxious adolescents. This would provide the opportunity to examine whether depression and anxiety contribute differently to the underlying

neurobiological mechanisms of these disorders. In this thesis a combined depression and anxiety group was included. We believe that a combined group is more ecologically valid since depression and anxiety are so closely related and comorbidity during adolescence is high (Essau, 2008). Because of this high relatedness of the two disorders, we used a dimensional approach by examining individual differences in amygdala activation and connectivity while taking self-reported depression and anxiety symptoms into account. This can provide us with important information about whether depression or anxiety has more influence on differentiating patterns of amygdala activation or connectivity. For example we showed a strong positive relation between the amount of amygdala activation during emotional face processing and the level of self-reported anxiety symptoms ***(chapter 2)***. We also described a relation between change in amygdala – dorsomedial prefrontal cortex connectivity and change in self-reported depression symptoms ***(chapter 6)***. These results suggest that depression and anxiety symptoms contribute differently to the underlying neurobiological mechanisms of adolescent depression and anxiety. By including larger samples of depressed and anxious adolescents, and thereby increasing power, the unique contribution of depression and anxiety symptoms can be further examined.

To conclude, the studies described in this thesis contain valuable new information about the neurobiological mechanisms of adolescent depression and anxiety disorders. Future research should extent the findings of this thesis by conducting large longitudinal studies with a pre-, post- and follow-up measurement. Multiple forms of treatment should be included (CBT-based as well as medication) and there should be a focus on individual differences in depression and anxiety symptoms. This kind of research will increase our knowledge on the neurobiological mechanisms of depression and anxiety disorders and will eventually lead to a starting point for the improvement of intervention and treatment strategies.

Nederlandstalige samenvatting

Inleiding

De overgang van de kindertijd naar de adolescentie is een gevoelige periode waarin veel veranderingen plaatsvinden: adolescenten gaan van de basisschool naar de middelbare school, ze beginnen vaak aan een bijbaantje, ze krijgen nieuwe vrienden die erg belangrijk voor ze worden en tegelijkertijd worden ze minder afhankelijk van hun ouders. Naast deze externe veranderingen, vinden er ook veel veranderingen plaats op biologisch niveau, zoals een toename in puberteitshormonen (Blakemore, Burnett, & Dahl, 2010) en de continuerende ontwikkeling van het brein (Giedd et al., 1999). De meeste adolescenten merken relatief weinig van deze veranderingen en de veranderingen zorgen niet voor grote problemen in hun verdere leven. Natuurlijk ervaren de meeste adolescenten wel wat problemen, zoals het vaker ruzie maken met hun ouders bijvoorbeeld over hoe laat ze thuis moeten zijn of het hebben van liefdesverdriet nadat hun eerste vriendje of vriendinnetje de verkering heeft uitgemaakt.

Bij de meeste adolescenten gaan deze gevoelens van boosheid, verdriet of somberheid naar verloop van tijd vanzelf weer over. Echter, bij sommige adolescenten verloopt de overgang van de kindertijd naar de adolescentie en de volwassenheid niet zonder gevolgen. Deze adolescenten ontwikkelen tijdens de adolescentie bijvoorbeeld een depressie of angststoornis. Hoewel we vrij veel weten over de ontwikkeling en instandhouding van depressie en angststoornissen tijdens de adolescentie en er verschillende behandelmethoden beschikbaar zijn die voor veel adolescenten effectief gebleken zijn (Compton, Burns, Helen, & Robertson, 2002; Compton, March, Brent, Albano, Weersing, & Curry, 2004), zijn er ook vrij veel adolescenten die niet profiteren van de bestaande behandelmethoden.

Door onderzoek te doen naar de onderliggende neurobiologische mechanismen van depressie en angststoornissen bij adolescenten, kunnen we beter inzicht krijgen in de ontwikkeling en instandhouding van deze stoornissen. Deze kennis kan mogelijk in de toekomst omgezet worden in betere interven-

tie en behandelstrategieën die beter aansluiten bij adolescenten.

In de afgelopen jaren zijn er verschillende studies uitgevoerd die aan hebben getoond dat de amygdala een belangrijk hersengebied is voor het verwerken van emoties (Fusar-Poli et al., 2009; Whalen, Davis, Oler, Kim, Kim, & Neta, 2009). Daarnaast heeft onderzoek aangetoond dat volwassenen en adolescenten met een depressie of angststoornissen meer amygdala activiteit laten zien tijdens het verwerken van emotionele gezichten dan gezonde controle proefpersonen (Beesdo et al., 2009b; Monk et al., 2008a; Monk et al., 2008b; Perlman et al., 2012; Thomas et al., 2001a). De resultaten van deze studies suggereren dat de amygdala een belangrijk gebied is om verder te onderzoeken als het gaat om de onderliggende neurobiologische mechanismen van depressie en angststoornissen.

Op basis van deze kennis, hebben we een grote longitudinale studie opgezet waarin we amygdala activiteit en connectiviteit hebben onderzocht in een groep adolescenten met een depressie en/of angststoornis, EPISCA (Emotional Pathways' Imaging Study in Clinical Adolescents). Binnen de studie was er sprake van drie hoofddoelen: (1) onderzoeken of adolescenten met depressie en/of angststoornis andere patronen van amygdala activiteit laten zien dan adolescenten zonder psychische klachten tijdens het verwerken van emotionele gezichten, (2) de test-hertest betrouwbaarheid van het fMRI-signaal te onderzoeken in verschillende hersengebieden die betrokken zijn bij het verwerken van emotionele gezichten en (3) de longitudinale veranderingen in amygdala activiteit en connectiviteit te onderzoeken bij adolescenten met depressie en/of angststoornis die cognitieve gedragstherapie (CGT) hebben gekregen.

In deze samenvatting worden kort de resultaten besproken van de verschillende studies uit dit proefschrift, waarin de adolescenten met depressie en angststoornissen vergeleken worden met normaal ontwikkelende adolescenten zonder psychische klachten (controlegroep). De samenvatting eindigt met het bespreken van de beperkingen van deze studies en de aanbevelingen voor toekomstig onderzoek.

Amygdala activiteit in reactie op emotionele gezichten

In ***hoofdstuk 2***, is een studie beschreven waarin onderzocht is of adolescenten met depressie en angststoornissen andere patronen van amygdala activiteit laten zien dan adolescenten zonder psychische klachten (controlegroep) tijdens het verwerken van emotionele gezichten. Op basis van de bestaande literatuur werd verwacht dat adolescenten met een DSM-IV diagnose van een depressie of angststoornis meer amygdala activiteit zouden laten zien bij het zien van angstige gezichten, dan de adolescenten uit de controle groep. Daarnaast verwachtten we dat er een positieve relatie zou zijn tussen de hoeveelheid zelf gerapporteerde angstsymptomen en activiteit in de amygdala. De resultaten van deze studie toonden aan dat de gebruikte taak voor activiteit zorgde in hersengebieden die eerder gekoppeld zijn aan het verwerken van emotionele gezichten, zoals de dorsolaterale prefrontale cortex (DLPFC), de amygdala en de visuele cortex (Costafreda et al, 2008; Fusar-Poli et al, 2009). Wanneer het patroon van hersenactiviteit tussen de twee groepen met elkaar vergeleken werd, werden er geen significante verschillen in hersenactiviteit gevonden. Als er in een meer specifiek gedeelte van de hersenen gekeken werd, de linker en rechter amygdala, konden er ook geen significante groepsverschillen gevonden worden. Wel was er een effect van emotie: de amygdala was meer actief tijdens het zien van bange en blije gezichten dan tijdens het zien van neutrale gezichten. Daarnaast werd een sterke positieve relatie gevonden tussen de hoeveelheid zelf gerapporteerde angstsymptomen en de mate van amygdala activiteit tijdens het zien van emotionele gezichten (bang, blij en neutraal) binnen de groep adolescenten met een depressie of angststoornis. Deze bevinding komt overeen met de resultaten van eerdere studies waarin ook een positieve relatie gevonden werd tussen de hoeveelheid zelf gerapporteerde angstsymptomen en amygdala activiteit tijdens het verwerken van emotionele gezichten (Bal et al, 2012; Monk et al, 2003a; Stein et al, 2007; Thomas et al, 2001a).

Hoewel er geen groepsverschillen werden gevonden in amygdala activiteit, suggereert de sterke positieve relatie tussen de hoeveelheid zelf

gerapporteerde angstsymptomen en amygdala activiteit dat angstsymptomen een onderliggende kenmerken eigenschap zijn voor zowel depressie als angststoornissen.

Vervolgens hebben we onderzocht of amygdala activiteit tijdens het verwerken van emotionele gezichten een verschillend patroon van habituatie laat zien bij adolescenten met depressie en angststoornissen, adolescenten die seksueel misbruikt zijn in de kindertijd en adolescenten uit de controle groep ***(hoofdstuk 3)***. Eerder onderzoek heeft aangetoond dat zowel adolescenten met depressie en angststoornissen als seksueel misbruikte adolescenten andere patronen van amygdala activiteit laten zien dan adolescenten zonder psychische klachten (Garrett et al, 2012; Monk et al, 2008b; Roberson-Nay et al, 2006). Daarnaast heeft onderzoek aangetoond dat habituatie van amygdala activiteit bij adolescenten met depressie of angststoornissen trager verloopt dan bij gezonde controle deelnemers (Hare et al., 2008). Op basis van deze eerdere studies verwachtten wij dat de adolescenten in de twee klinische groepen een andere mate van habituatie van amygdala activiteit zouden laten zien dan de adolescenten uit de controle groep. Daarnaast waren we geïnteresseerd in de verschillen in habituatie tussen de depressie/angst groep en de groep adolescenten die seksueel misbruik hebben meegemaakt. Adolescenten in deze twee klinische groepen laten een grote overlap in symptomen zien, maar de adolescenten uit de seksueel misbruik groep hebben ook iets unieks, namelijk het meemaken van een traumatische ervaring.

De resultaten van deze studie toonden aan dat het patroon van amygdala habituatie in de controle groep verschilde van het patroon van habituatie in de twee klinische groepen. De depressieve en angstige adolescenten lieten aan het begin van de taak een vergelijkbaar patroon van amygdala activiteit zien als de controle groep, maar er was geen sprake van habituatie van amygdala activiteit in deze groep terwijl de adolescenten uit de controle groep wel habituatie van amygdala activiteit lieten zien. De ado-

lescenten die seksueel misbruik hebben meegemaakt lieten een heel ander patroon van amygdala habituatie zien: zij vertoonden meer amygdala activiteit tijdens het eerste gedeelte van de taak, gevolgd door een snelle habituatie van amygdala activiteit tot een niveau vergelijkbaar met dat van de twee andere groepen. Mogelijk is de initiële verhoogde amygdala activiteit in de groep adolescenten die seksueel misbruik hebben meegemaakt, gerelateerd aan een verhoogde waakzaamheid voor emotionele gezichten welke veroorzaakt kan zijn door het meemaken van een traumatische ervaring. Daarnaast lijkt de regulerende functie van cognitieve controle gebieden in de hersenen bij deze groep intact, aangezien er vrij snel habituatie van amygdala activiteit optreedt. Bij adolescenten met depressie en angststoornissen werkt het mechanismen mogelijk de andere kant op: bij hen lijkt er geen sprake te zijn van een verhoogde initiële emotionele reactie (verhoogde amygdala activiteit), maar mogelijk is de integratie van informatie door cognitieve controle gebieden onvoldoende wat kan leiden tot emotie regulatie problemen. Deze hypothese zou passen bij het top-down regulatie model voor depressie, waarin men stelt dat depressiesymptomen veroorzaakt en in stand gehouden worden door een inefficiënte top-down regulatie door de prefrontale cortex (Mayberg, 1997).

Betrouwbaarheid van het fMRI-signaal

Momenteel worden er steeds meer longitudinale studies opgezet waarin individuele verschillen in neurobiologische mechanismen worden onderzocht. Om de resultaten van deze longitudinale studies op de juiste manier te kunnen interpreteren, is het van belang om te weten of patronen van hersenactiviteit binnen proefpersonen gelijk is over tijd. De laatste jaren is er in beperkte mate onderzoek gedaan naar de test-hertest betrouwbaarheid van het fMRI signaal in gezonde volwassen proefpersonen (Johnstone et al, 2005; Plichta et al, 2012). Vergelijkbaar onderzoek bij adolescenten ontbreek echter, terwijl de adolescentie juist een periode is waarin aanzienlijke veranderingen optreden in het sociaal emotioneel functioneren (Dahl, 2004). Deze

veranderingen zijn mogelijk van invloed op de test hertest betrouwbaarheid van het fMRI-signaal.

In ***hoofdstuk 4*** wordt een studie beschreven waarin de test-hertest betrouwbaarheid van activiteit in verschillende hersengebieden is onderzocht. Alle beschreven hersengebieden zijn betrokken bij het verwerken van emotionele gezichten. We hebben gebruik gemaakt van een groep normaal ontwikkelende adolescenten zonder psychische klachten die drie keer werden gescand in een periode van zes maanden. De resultaten van laten zien dat de gebruikte taak hersengebieden activeerde die eerder gerelateerd zijn aan het verwerken van emotionele gezichten: bilaterale amygdala, bilaterale DLPFC en de visuele cortex. Daarnaast toonden de analyses van zowel de gedragsdata als de fMRI data geen significante veranderingen over tijd. Echter, als er gebruikt werd gemaakt van een specifieke statistische methode, test-hertest betrouwbaarheidsanalyses, bleek dat er aanzienlijke intra-individuele variatie is in DLPFC en amygdala activiteit: de test-hertest betrouwbaarheid voor de visuele cortex was hoog, die voor de DLPFC redelijk en voor de amygdala was deze laag. Deze bevindingen tonen aan dat er zeker sprake kan zijn van intra-individuele variatie in hersenactiviteit over tijd, ook al wordt dit niet gevonden in groepsanalyses. In de groepsanalyses kan de individuele variatie over tijd uitgemiddeld worden door het samen nemen van alle data van alle proefpersonen. De bevindingen uit ***hoofdstuk 4*** komen overeen met de resultaten van eerdere studies waarin de test-hertest betrouwbaarheid van hersenactiviteit is onderzocht in volwassenen (Plichta et al., 2012). Het is belangrijk dat toekomstige longitudinale onderzoeken de huidige bevindingen meenemen bij het interpreteren van hun bevindingen.

Longitudinale veranderingen in de amygdala activiteit

Eerdere studies hebben aangetoond dat adolescenten met depressie en angststoornissen andere patronen van amygdala activiteit laten zien dan adolescenten zonder psychische klachten (Monk et al, 2008a; Monk et al,

2008b; Perlman et al, 2012; Thomas et al., 2001a). Echter, dit soort studies bevatten vaak maar één meting. hierdoor is het niet mogelijk om de longitudinale veranderingen in de onderliggende neurobiologische mechanismen van depressie en angst te onderzoeken. Er zijn een aantal studies die proefpersonen met een depressie en/of angststoornis over tijd hebben gevolgd, maar deze studies hebben vaak maar één fMRI meting bij de start van de (Canli et al, 2005; Siegle et al, 2006). De resultaten van deze studies suggereren dat de hoeveelheid amygdala activiteit in reactie op emotionele gezichten af neemt als gevolg van de behandeling die de proefpersonen kregen (Clark & Beck, 2010; Guide et al, 2012). Opvallend is dat deze studies eigenlijk alleen maar gedaan zijn met volwassen proefpersonen. Er is slechts één longitudinale studie waarbij de onderzoekers gekeken hebben naar veranderingen in amygdala activiteit in een groep adolescenten met angststoornis. De resultaten van deze studie lieten een toename in amygdala activiteit over tijd zien in een gedeelte van de groep proefpersonen, namelijk de adolescenten die een doorverwijzing ontvingen van behandeling met CGT (Maslowsky et al., 2010).

Om een beter beeld te krijgen van eventuele longitudinale veranderingen in amygdala activiteit bij adolescenten met een depressie of angststoornis, hebben we een longitudinale fMRI studie uitgevoerd. In deze studie werden adolescenten met een depressie en/of angststoornis en normaal ontwikkelende adolescenten zonder psychische klachten drie keer gescand in een periode van zes maanden ***(hoofdstuk 5)***. Tijdens elke scansessie maakten de adolescenten een emotionele gezichten taak waarin zij bange, blije en neutrale gezichten te zien kregen. De resultaten van deze studie lieten zien dat de adolescenten uit de klinische groep een significante afname liet zien in zelf gerapporteerde depressie en angstsymptomen. Daarnaast kwam naar voren dat de adolescenten uit de klinische groep op de derde meting significant meer amygdala activiteit lieten zien dan de adolescenten uit de controle groep. Op de eerste en de tweede meting was er geen significant verschil tussen de groepen, wat een toename van amygdala activiteit over

tijd suggereert in de klinische groep. Deze bevindingen komen overeen met de eerdere bevindingen van Maslowsky en collega's (2010): zij rapporteerde een toename in amygdala activiteit over tijd in een groep adolescenten met een angststoornis die CGT hebben gekregen. Daarnaast zijn er recente studies die aantonen dat het uitdoven van een geconditioneerde angst reactie bij adolescenten veel langer duurt dan bij kinderen en volwassenen, wat zou kunnen leiden tot een verhoogde gevoeligheid van de amygdala in reactie op emotionele stimuli (Drysdale et al, 2013; Pattwell et al, 2012). Toekomstig onderzoek is nodig om de robuustheid van deze effecten verder te onderzoeken en om te bepalen of de longitudinale veranderingen samenhangen met veranderingen in de hoeveelheid zelf gerapporteerde symptomen en/of behandeluitkomst.

Naast het onderzoeken van longitudinale veranderingen in taak gerelateerde amygdala activiteit, hebben we ook gekeken naar longitudinale veranderingen in functionele connectiviteit tijdens rust. Eerder onderzoek heeft aangetoond dat adolescenten met depressie en angststoornissen andere patronen van functionele connectiviteit laten zien dan normaal ontwikkelende adolescenten zonder psychische klachten tijdens rust. Deze verschillen werden bijvoorbeeld gevonden in de connectiviteit tussen de amygdala en delen van de mediale prefrontale cortex (Hulvershorn et al, 2011; Pannekoek et al, 2014a). Deze studies bevatten meestal maar één meting en het daardoor niet mogelijk om te kijken naar longitudinale veranderingen in functionele connectiviteit tijdens rust in deze hersengebieden. In ***hoofdstuk 6*** wordt een studie beschreven waarin longitudinale veranderingen in functionele connectiviteit tijdens rust onderzocht zijn. Daarbij is een groep adolescenten met depressie en angststoornissen vergeleken met een controle groep. Alle adolescenten zijn twee keer gescand in een periode van zes maanden. Gedurende deze zes maanden werden de adolescenten met depressie en angststoornissen behandeld voor hun klachten door middel van CGT. We hebben specifiek gekeken naar functionele connectiviteit vanuit de

amygdala (bilateraal) naar andere hersengebieden. De resultaten lieten een significante interactie tussen groep en sessie zien, waarbij de adolescenten met een depressie en angststoornis een toename in positieve connectiviteit lieten zien tussen de rechter amygdala en de mediale prefrontale cortex. Daarnaast was er een significante negatieve relatie tussen de verandering rechter amygdala – mediale prefrontale cortex connectiviteit en verandering in zelf gerapporteerde depressiesymptomen in de hele groep proefpersonen. Met andere woorden: adolescenten die een sterkere toename van positieve connectiviteit tussen de amygdala en mediale prefrontale cortex lieten zien, rapporteerden ook een grotere afname in depressiesymptomen.

Hoewel de gebruikte functionele connectiviteitsanalyses geen informatie geven over de richting van de effecten, kunnen de resultaten mogelijk wijzen op een toename in top-down regulatie van de mediale prefrontale cortex over de amygdala. Deze interpretatie komt overeen met een veel gebruikt model voor depressie waarin gesuggereerd wordt dat depressiesymptomen worden veroorzaakt door een inefficiënte top-down regulatie van de prefrontale cortex over de primaire emotionele reactie van de amygdala (Mayberg, 1997). Andere studies die functionele connectiviteit tijdens rust hebben onderzocht in volwassenen met depressie en angststoornissen ondersteunen dit model (Clark, & Beck, 2010; Månsson et al, 2013). Hoewel de huidige bevindingen ons nieuwe inzichten geven in de verandering in functionele connectiviteit tijdens rust bij adolescenten met depressie en angststoornissen, is verder onderzoek nodig. Daarbij is het belangrijk dat onderzoekers in de opzet van hun studie verschillende vormen van behandeling verwerken, zoals een gestructureerde vorm van CGT of specifieke medicatie.

Conclusie

Het doel van de beschreven studies in dit proefschrift was om de neurobiologische mechanismen van depressie en angststoornissen in de adolescentie te onderzoeken. Hiervoor is gebruik gemaakt van een cross-sectionele en longitudinale studie opzet en er is gekeken naar zowel taak ge-

relateerde hersenactiviteit als functionele connectiviteit tijdens rust. De resultaten van de verschillende studies hebben aangetoond dat adolescenten met depressie en angststoornissen andere patronen van amygdala activiteit en connectiviteit laten zien dan normaal ontwikkelende adolescenten zonder psychische klachten. De bevindingen hebben aangetoond dat de amygdala een belangrijk hersengebied is voor het verwerken van emotionele gezichten en dat een focus op amygdala activiteit en connectiviteit verder inzicht kan geven in het ontstaan van depressie en angststoornissen tijdens de adolescentie. Daarnaast bieden de longitudinale studies handvaten voor toekomstig onderzoek naar het verloop en de in stand houding van depressie en angststoornissen tijdens de adolescentie. In alle beschreven studies van dit proefschrift is gebruik gemaakt van een dimensionele benadering in plaats van een categorische benadering. Met andere woorden, er is gekeken naar het verloop in zelf gerapporteerde depressie en angstsymptomen in plaats van alleen maar naar het wel of niet hebben van een depressie of angststoornis. Uit deze dimensionele benadering kwam naar voren dat vooral zelf gerapporteerde angstsymptomen een belangrijke rol lijken te spelen in de mate van amygdala activiteit in reactie op emotionele gezichten.

In de volgende paragrafen zal ik een aantal algemene overwegingen en richtlijnen voor toekomstig onderzoek weergeven.

Groepsvergelijkingen

In de ***hoofdstukken 2 en 3*** beschrijven we de resultaten van twee studies met behulp van groepsvergelijkingen en gegevens van slechts één sessie. De resultaten van deze studies toonden een sterke positieve relatie tussen de hoeveelheid zelf gerapporteerde angstsymptomen en de mate van amygdala activiteit. Deze relatie werd niet gevonden voor zelf gerapporteerde depressiesymptomen. Daarnaast waren er verschillen in de mate van habituatie van amygdala activiteit tussen groepen: adolescenten met depressie en angststoornissen leken geen habituatie van amygdala activiteit te laten zien, terwijl adolescenten die seksueel misbruik hebben meegemaakt en

normaal ontwikkelende adolescenten wel habituatie van amygdala activiteit laten zien.

De specifieke relatie tussen amygdala activiteit en zelf gerapporteerde angstsymptomen duidt er mogelijk op dat angstsymptomen, en niet depressiesymptomen, een belangrijke voorspeller zijn voor onderscheidende patronen van amygdala activiteit in deze klinische groepen. Hoewel meerdere studies het belang laten zien van het meenemen van vragenlijsten voor zelf gerapporteerde depressie en angstsymptomen om onderscheidende patronen van amygdala activiteit te onderzoeken, zijn er niet heel veel studies die dit ook daadwerkelijk hebben gedaan. Daarnaast lijkt er wat inconsistentie te zijn over de relatie van zelf gerapporteerde depressie en angstsymptomen en amygdala activiteit: een aantal studies laat consistent zien dat adolescenten die meer angst rapporteren ook meer amygdala activiteit laten zien tijdens een emotionele gezichten taak (McClure et al., 2007b; Monk et al, 2008b), terwijl vergelijkbare studies waarin adolescenten met een depressie stoornis worden onderzocht vaak een inconsistent beeld geven (Monk et al, 2008a; Roberson-Nay et al, 2006; Thomas et al, 2001a). Om meer duidelijkheid te krijgen over de exacte relatie tussen zelf gerapporteerde angstsymptomen en amygdala activiteit in zowel adolescenten met depressie als adolescenten met angststoornissen, moet meer onderzoek gedaan worden waarin een dimensionele benadering gebruikt wordt.

Zoals beschreven in ***hoofdstuk 3***, lijken de adolescenten met depressie en angststoornissen geen habituatie van amygdala activiteit te vertonen tijdens het zien van emotionele gezichten. Het ontbreken van habituatie in deze groep en de snelle habituatie in de groep adolescenten die seksueel misbruik hebben meegemaakt, wijst mogelijk op een verschillend onderliggend neurobiologisch mechanisme voor deze twee klinische groepen. Hoewel speculatief, kunnen de resultaten wijzen op een predispositie voor het ontwikkelen van depressie en angststoornissen door de aanwezigheid van specifieke persoonlijkheidsstijlen, zoals neuroticisme. De ontwikkeling van depressie en angstsymptomen bij adolescenten die seksueel misbruik heb-

ben meegemaakt lijkt daarentegen per definitie het gevolg van een traumatische ervaring: mogelijk maakt het meemaken van een traumatische ervaring deze adolescenten meer waakzaam voor emotionele gezichten wat tot uiting komt in verhoogde amygdala activiteit tijdens het verwerken van emotionele gezichten (Garrett et al, 2012; Hart, & Rubia, 2012). Voor adolescenten met depressie en angststoornissen werkt dit mechanisme misschien anders: mogelijk liggen de problemen in deze groep niet bij een verhoogde primaire reactie op emotionele gezichten (amygdala activiteit), maar in de verminderde top-down regulatie door de prefrontale cortex welke de depressie en angstsymptomen kunnen verminderen. Mogelijk kan toekomstig onderzoek meer duidelijkheid geven over de onderliggende neurobiologische mechanismen door bijvoorbeeld naar hersen activiteit te kijken terwijl proefpersonen actief proberen hun emoties en gevoelens te reguleren.

Longitudinale veranderingen

In de ***hoofdstukken 5 en 6*** zijn de longitudinale veranderingen in amygdala activiteit en connectiviteit onderzocht. Voor zowel amygdala activiteit als amygdala connectiviteit vonden we significante veranderingen over tijd. Binnen de groep adolescenten met depressie en angststoornissen vonden we een toename in amygdala activiteit over tijd in reactie op het verwerken van emotionele gezichten. Ook vonden we in deze groep een toename in positieve connectiviteit tussen de rechter amygdala en de mediale prefrontale cortex.

De toename in amygdala activiteit over tijd tijdens het verwerken van emotionele gezichten hebben we geïnterpreteerd als zijnde een verhoogde gevoeligheid van amygdala activiteit die mogelijk gedreven wordt door de invloed van behandeling of door de verandering in de hoeveelheid klachten. Eerder onderzoek door Maslowsky en collega's (2010) liet vergelijkbare resultaten zien, namelijk een toename in amygdala activiteit in een groep adolescenten die behandeld werden met CGT. Recent zijn er een aantal studies verschenen die deze bevindingen ondersteunen. Deze studies suggereren dat adolescenten er langer over doen om een aangeleerde angstreactie weer

te laten uitdoven. Dit kan dan weer leiden tot een verhoogde gevoeligheid van de amygdala in reactie op emotionele gezichten (Drysdale et al, 2013; Pattwell et al, 2012). De resultaten voor de connectiviteitsanalyses, waarbij een toename in positieve connectiviteit tussen de amygdala en de mediale prefrontale cortex werd gevonden, zijn geïnterpreteerd als een toename in top-down controle van de mediale prefrontale cortex over de amygdala. Deze interpretatie past in de bestaande literatuur over vergelijkbaar onderzoek bij volwassenen (Fu et al, 2008; Månsson et al, 2013) en bij de huidige ideeën over de onderliggende neurobiologische mechanismen van depressie en angststoornissen (Mayberg, 1997; Quide et al, 2012).

Als we resultaten van deze twee studies samenvoegen, dan lijken zij elkaar te ondersteunen: de toename in positieve connectiviteit tussen de amygdala en de mediale prefrontale cortex kan worden aangedreven door een toename van de primaire reactie in de amygdala. Dit kan vervolgens leiden tot een verhoogde top-down regulatie door de mediale prefrontale cortex. De functionele connectiviteitsanalyses geven echter geen informatie over de richting van de toename in positieve connectiviteit. Het is op basis van de huidige bevindingen niet te zeggen of amygdala activiteit meer op mediale prefrontale cortex activiteit gaat lijken of andersom. Om hier verdere uitspraken over te doen, zijn complexe analyse methoden nodig zoals Dynamic Causal Modelling (DCM) (Friston, Harrison & Penny, 2003). Het zou zeker interessant zijn om in toekomstig longitudinaal onderzoek deze twee methoden, taak gerelateerde amygdala activiteit en amygdala connectiviteit, te combineren en verder te onderzoeken. Mogelijk geeft ons dat dan meer informatie over wat er precies gebeurt in de hersenen van adolescenten met depressie en angststoornissen; is er een toename in amygdala activiteit, een toename in top-down regulatie vanuit de mediale prefrontale cortex en/of van beide mechanismen? Daarnaast kan dergelijk longitudinaal onderzoek de relatie tussen veranderingen in de hersenen, veranderingen in zelf gerapporteerde depressie en angstsymptomen en behandeleffectiviteit verder onderzoeken.

In ***hoofdstuk 4*** beschrijven we een longitudinale studie waarin we de test-hertest betrouwbaarheid van het fMRI signaal hebben onderzoek in een groep normaal ontwikkelende adolescenten. De resultaten van deze studie toonden aan dat de test-hertest betrouwbaarheid van de visuele cortex heel goed is, van de laterale prefrontale cortex matig is en van de amygdala heel slecht is. Met andere woorden, de activiteit van de visuele cortex is binnen proefpersonen heel stabiel over tijd, terwijl de activiteit van de amygdala binnen proefpersonen heel erg kan verschillen over tijd. Deze resultaten komen overeen met eerder onderzoek waarin de betrouwbaarheid van vergelijkbare gebieden getest is in volwassenen (Hare et al, 2008; Plichta et al, 2012.). Het is erg belangrijk dat onderzoekers rekening houden met deze variatie van activiteit in bepaalde gebieden binnen proefpersonen, vooral wanneer er drie of meer metingen gedaan worden. De momenteel beschikbare analyse programma's voor fMRI data, zoals SPM (Statistical Parametric Mapping; Welcome Department Cognitive Neurology, Londen), beschikken nog niet over de best passende analyse methoden voor het analyseren van longitudinale hersen data. Bij deze methoden moet men denken aan bijvoorbeeld multi-level modellen. Een model dat momenteel wel al beschikbaar is, is het 'flexible factorial model'. Binnen dit model bestaat de mogelijkheid om aan te geven dat proefpersonen meerdere keren zijn getest en dat de variabiliteit over tijd binnen proefpersonen in acht moet worden genomen. Echter, niet alle beschikbare modellen voor hersenanalyses bieden deze flexibiliteit. Als er analyses gedaan worden op basis van specifieke interesse gebieden met behulp van statistische programma's zoals SPSS, dan zijn er al veel meer dingen mogelijk: bijvoorbeeld het gebruik herhaalde metingen ANOVA of multilevel analyse. Hopelijk leidt toekomstig onderzoek tot de beschikbaarheid van betere statistische modellen in de analyse programma's voor fMRI zodat longitudinale analyse nog beter uitgevoerd kunnen worden.

Beperkingen en aanbevelingen

Hoewel de bevindingen uit dit proefschrift interessant zijn en ons nieuwe informatie geven over de ontwikkeling en in stand houding van depressie en angststoornissen in de adolescentie, is het van belang dat andere onderzoeksgroepen deze resultaten repliceren. Daarbij moeten een aantal punten meegenomen worden in de opzet van de studies. Allereerst adviseren wij dat onderzoekers gebruik maken van een longitudinale studie opzet waarbij er sprak is van een voormeting, een meting na behandeling en een follow-up meting. Door een gebruik te maken van een dergelijke opzet, is het mogelijk om met meer zekerheid conclusies te trekken over de veranderingen in bijvoorbeeld hersenactiviteit en de relatie met behandel effecten. Daarnaast geeft het onderzoekers de mogelijkheid om uitspraken te doen over welke jongeren wel profiteren van behandeling en welke jongeren niet. In de studieopzet die gebruikt is voor dit proefschrift was deze opzet niet haalbaar: door gebruik te maken van 'treatment as usual' ontstond er een grote variabiliteit in het aantal behandelingen, de periode waarin de adolescenten behandeling kregen en de precieze invulling van de behandelingen. Alle behandelingen hadden als basis CGT, maar sommige adolescenten ontvingen individuele therapie, terwijl andere adolescenten groepstherapie hebben gekregen.

In aanvulling op deze suggesties, is het ook van belang dat toekomstig onderzoek gebruik maakt van een gestandaardiseerde vorm van behandeling. Gedacht kan worden aan het gebruik van een gestructureerd CGT protocol waarbij alle adolescenten evenveel behandel sessies krijgen die op dezelfde manier ingevuld worden. Tevens zou het interessant zijn om meerdere behandelvormen met elkaar te vergelijken. Hierbij kan gedacht worden aan een groep adolescenten die CGT ontvangen, een groep adolescenten die medicatie ontvangen en een groep adolescenten die beide vormen van behandeling ontvangen. Onderzoek heeft namelijk aangetoond dat zowel CGT als medicatie effectief kan zijn in de behandeling van depressie en angststoornissen en dat een combinatie van beide behandelmethoden mogelijk

nog effectiever is (Compton et al, 2004; Walkup et al., 2008). Het is echter niet duidelijk in hoeverre deze vormen van behandeling de onderliggende neurobiologische mechanismen van depressie en angststoornissen tijdens de adolescentie beïnvloeden.

Tenslotte is het belangrijk dat toekomstig onderzoek er naar streeft om grotere groepen adolescenten met depressie en angststoornissen probeert te includeren. Door meer proefpersonen te includeren, is het mogelijk om nog specifieker te kijken naar mogelijke verschillen in de neurobiologische mechanismen tussen depressie en angststoornissen. In het onderzoek dat beschreven is in dit proefschrift hebben we gebruik gemaakt van een gecombineerde groep adolescenten met een depressie en/of angststoornissen. Wij zijn van mening dat een gecombineerde groep een betere representatie van wat clinici in de praktijk tegen komen. Depressie en angststoornissen zijn vooral tijdens de adolescentie zo nauw aan elkaar verwant (hoge co morbiditeit) dat het bijna onmogelijk is om de stoornissen geheel los van elkaar te zien (Essau, 2008). Om toch uitspraken te kunnen doen over de invloed van depressie en angststoornissen op bijvoorbeeld amygdala activiteit, hebben we een dimensionele benadering gebruikt. Deze benadering kan ons belangrijke informatie geven over de vraag of depressie of juist angststoornissen meer invloed hebben op onderscheidende patronen van amygdala activiteit en connectiviteit. We hebben bijvoorbeeld een sterk positief verband gevonden tussen de hoeveelheid amygdala activiteit tijdens het verwerken van emotionele gezichten en de hoeveelheid zelf gerapporteerde angstsymptomen (***hoofdstuk 2***). Daarnaast hebben we een relatie gevonden tussen de verandering in amygdala - mediale prefrontale cortex activiteit en zelf gerapporteerde depressiesymptomen (***hoofdstuk 6***). Deze resultaten suggereren dat depressie en angstsymptomen op een andere manier invloed hebben op de onderliggende neurobiologische mechanismen van depressie en angst. Door het includeren van meer proefpersonen van depressie en angststoornissen, waarmee je de power van de analyses vergroot, kan de unieke bijdrage van depressie en angstsymptomen verder onderzocht worden.

Concluderend kan gezegd worden dat de beschreven studies in dit proefschrift belangrijke en waardevolle nieuwe informatie bevatten over de onderliggende neurobiologische mechanismen van depressie en angststoornissen bij adolescenten. Toekomstig onderzoek moet de huidige bevindingen zeker proberen te repliceren en uit te breiden door gebruik te maken van grote longitudinale studies met een voor-, na- en follow-up meting waarin adolescenten met een depressie en angststoornis een gestructureerde vorm van behandeling aangeboden krijgen. Alleen door het doen van dit soort studies, kunnen we onze kennis over de ontwikkeling en instandhouding van depressie en angststoornissen tijdens de adolescentie vergroten. In het meest ideale geval zal dat uiteindelijk leiden tot een verbetering van de huidige interventie en behandelstrategieën.

References

Adolphs, R. (2002). Recognizing emotion from facial expressions: Psychological and neurological mechanisms. Behav.Cogn Neurosci.Rev., 1, 21-62

Adolphs, R., Tranel, D., & Damasio, A. R. (2003). Dissociable neural systems for recognizing emotions. Brain Cogn, 52, 61-69

Aghajani, M., Veer, I. M., Van Lang, N. D., Meens, P. H., Van Den Bulk, B. G., Rombouts, S. A., Vermeiren, R. R., & Van Der Wee, N. J. (2013). Altered white-matter architecture in treatment-naive adolescents with clinical depression. Psychol Med, 1-12

Anand, A., Li, Y., Wang, Y., Wu, J., Gao, S., Bukhari, L., Mathews, V. P., Kalnin, A., & Lowe, M. J. (2005). Activity and connectivity of brain mood regulating circuit in depression: A functional magnetic resonance study. Biol Psychiatry, 57, 1079-1088

Aron, A. R., Gluck, M. A., & Poldrack, R. A. (2006). Long-term test-retest reliability of functional mri in a classification learning task. Neuroimage, 29, 1000-1006

Baird, A. A., Gruber, S. A., Fein, D. A., Maas, L. C., Steingard, R. J., Renshaw, P. F., Cohen, B. M., & Yurgelun-Todd, D. A. (1999). Functional magnetic resonance imaging of facial affect recognition in children and adolescents. J Am Acad Child Adolesc Psychiatry, 38, 195-199

Ball, T. M., Sullivan, S., Flagan, T., Hitchcock, C. A., Simmons, A., Paulus, M. P., & Stein, M.B. (2012). Selective effects of social anxiety, anxiety sensitivity, and negative affectivity on the neural bases of emotional face processing. Neuroimage, 59, 1879-1887

Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., & Van Ijzendoorn, M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: A meta-analytic study. Psychol Bull, 133, 1-24

Beesdo, K., Knappe, S., & Pine, D. S. (2009a). Anxiety and anxiety disorders in children and adolescents: Developmental issues and implications for dsm-v. Psychiatr Clin North Am, 32, 483-524

Beesdo, K., Lau, J. Y. F., Guyer, A. E., Mcclure-Tone, E. B., Monk, C. S., Nelson, E. E., Fromm, S. J., Goldwin, M. A., Wittchen, H.-U., Leibenluft, E., Ernst, M., & Pine, D. S. (2009b). Common and distinct amygdala-function perturbations in depressed vs anxious adolescents. Arch. Gen. Psychiatry, 66, 275-285

Bennett, C. M., & Miller, M. B. (2010). How reliable are the results from functional magnetic resonance imaging? Ann N Y Acad Sci, 1191, 133-155

Biswal, B., Yetkin, F. Z., Haughton, V. M., & Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar mri. Magn Reson.Med., 34, 537-541

Blair, K. S., Geraci, M., Smith, B. W., Hollon, N., Devido, J., Otero, M., Blair, J. R., & Pine, D. S. (2012). Reduced dorsal anterior cingulate cortical activity during emotional regulation and top-down attentional control in generalized social phobia, generalized anxiety disorder, and comorbid generalized social phobia/generalized anxiety disorder. Biol Psychiatry, 72, 476-482

Blakemore, S. J., Burnett, S., & Dahl, R. E. (2010). The role of puberty in the developing adolescent brain. Hum. Brain Mapp, 31, 926-933

Blakemore, S. J. (2012). Imaging brain development: The adolescent brain. Neuroimage, 61, 397-406

Breiter, H. C., Etcoff, N. L., Whalen, P. J., Kennedy, W. A., Rauch, S. L., Buckner, R. L., Strauss, M. M., Hyman, S. E., & Rosen, B. R. (1996). Response and habituation of the human amygdala during visual processing of facial expression. Neuron, 17, 875-887

Brett, M., Johnsrude, I. S., & Owen, A. M. (2002). The problem of functional localization in the human brain. Nat.Rev.Neurosci., 3, 243-249

Brotman, M. A., Rich, B. A., Schmajuk, M., Reising, M., Monk, C. S., Dickstein, D. P., Mogg, K., Bradley, B. P., Pine, D. S., & Leibenluft, E. (2007). Attention bias to threat faces in children with bipolar disorder and comorbid lifetime anxiety disorders. Biol Psychiatry, 61, 819-821

Caceres, A., Hall, D. L., Zelaya, F. O., Williams, S. C., & Mehta, M. A. (2009). Measuring fmri reliability with the intra-class correlation coefficient. Neuroimage, 45, 758-768

Canli, T., Cooney, R. E., Goldin, P., Shah, M., Sivers, H., Thomason, M. E., Whitfield Gabrieli, S., Gabrieli, J. D., & Gotlib, I. H. (2005). Amygdala reactivity to emotional faces predicts improvement in major depression. Neuroreport, 16, 1267-1270

Casey, B., Jones, R. M., & Somerville, L. H. (2011). Braking and accelerating of the adolescent brain. J Res Adolesc, 21, 21-33

Casey, B. J., Jones, R. M., & Hare, T. A. (2008). The adolescent brain. Ann N Y Acad Sci, 1124, 111-126

Chorpita, B. F., Yim, L., Moffitt, C., Umemoto, L. A., & Francis, S. E. (2000). Assessment of symptoms of dsm-iv anxiety and depression in children: A revised child anxiety and depression scale. Behav.Res.Ther., 38, 835-855

Cicchetti, D. V., & Sparrow, S. A. (1981). Developing criteria for establishing interrater reliability of specific items: Applications to assessment of adaptive behavior. Am J Ment Defic, 86, 127-137

Cicchetti, D. V. (2001). The precision of reliability and validity estimates re-visited: Distinguishing between clinical and statistical significance of sample size requirements. J Clin Exp Neuropsychol, 23, 695-700

Clark, D. A., & Beck, A. T. (2010). Cognitive theory and therapy of anxiety and depression: Convergence with neurobiological findings. Trends Cogn Sci, 14, 418-424

Clark, L. A., & Watson, D. (1991). Tripartite model of anxiety and depression: Psychometric evidence and taxonomic implication. Journal of abnormal psychology, 100, 316-336

Cocosco, C. A., Kollokian, V., Kwan, R. K. S., & Evans, A. C. (1997). Brainweb: Online interface to a 3d mri simulated brain database. Neuorimage, 5, s425

Compton, S. N., Burns, B. J., Helen, L. E., & Robertson, E. (2002). Review of the evidence base for treatment of childhood psychopathology: Internalizing disorders. J.Consult Clin.Psychol., 70, 1240-1266

Compton, S. N., March, J. S., Brent, D., Albano, A. M., Weersing, V. R., & Curry, J. (2004). Cognitive-behavioral psychotherapy for anxiety and depressive disorders in children and adolescents: An evidence-based medicine review. Journal of the American Academy of Child & Adolescent Psychiatry, 43, 930-959

Costafreda, S. G., Brammer, M. J., David, A. S., & Fu, C. H. (2008). Predictors of amygdala activation during the processing of emotional stimuli: A meta-analysis of 385 pet and fmri studies. Brain Res Rev, 58, 57-70

Costafreda, S. G., Khanna, A., Mourao-Miranda, J., & Fu, C. H. Y. (2009). Neural correlates of sad faces predict clinical remission to cognitive behavioural therapy in depression. Neuroreport, 20, 637-641

Costello, E. J., Copeland, W., & Angold, A. (2011). Trends in psychopathology across the adolescent years: What changes when children become adolescents, and when adolescents become adults? J Child Psychol Psychiatry, 52, 1015-1025

Crone, E. A., & Dahl, R. E. (2012). Understanding adolescence as a period of social affective engagement and goal flexibility. Nat.Rev.Neurosci., 13, 636-650

Crone, E. A., & Elzinga, B. M. (2014). Changing brains: How longitudinal fmri studies can inform us about cognitive and social-affective growth trajectories. Wire Cognitive Science,

Cullen, K. R., Gee, D. G., Klimes-Dougan, B., Gabbay, V., Hulvershorn, L., Mueller, B. A., Camchong, J., Bell, C. J., Houri, A., Kumra, S., Lim, K. O., Castellanos, F. X., & Milham, M. P. (2009). A preliminary study of functional connectivity in comorbid adolescent depression. Neurosci Lett, 460, 227-231

Cunningham, W. A., Van Bavel, J. J., & Johnsen, I. R. (2008). Affective flexibility: Evaluative processing goals shape amygdala activity. Psychol.Sci., 19, 152-160

Dahl, R. E. (2004). Adolescent development and the regulation of behavior and emotion: Introduction to part viii. Ann N Y Acad Sci, 1021, 294-295

Davis, M., & Whalen, P. J. (2001). The amygdala: Vigilance and emotion. Mol. Psychiatry, 6, 13-34

Del Barrio, V., Moreno-Rosset, C., Lopez-Martinez, R., & Olmedo, M. (1997). Anxiety, depression and personality structures. Personality and individual differences, 23, 327-335

Derntl, B., Windischberger, C., Robinson, S., Lamplmayr, E., Kryspin-Exner, I., Gur, R. C., Moser, E., & Habel, U. (2008). Facial emotion recognition and amygdala activation are associated with menstrual cycle phase. Psychoneuroendocrinology, 33, 1031-1040

Drysdale, A. T., Hartley, C. A., Pattwell, S. S., Ruberry, E. J., Somerville, L. H., Compton, S. N., Lee, F. S., Casey, B. J., & Walkup, J. T. (2013). Fear and anxiety from principle to practice: Implications for when to treat youth with anxiety disorders. Biol Psychiatry,

Eaton, K. P., Szaflarski, J. P., Altaye, M., Ball, A. L., Kissela, B. M., Banks, C., & Holland, S. K. (2008). Reliability of fmri for studies of language in post-stroke aphasia subjects. Neuroimage, 41, 311-322

Essau, C. A. (2003). Comorbidity of anxiety disorders in adolescents. Depress Anxiety, 18, 1-6

Essau, C. A. (2008). Comorbidity of depressive disorders among adolescents in community and clinical settings. Psychiatry Res, 158, 35-42

Fischer, H., Wright, C. I., Whalen, P. J., Mcinerney, S. C., Shin, L. M., & Rauch, S. L. (2003). Brain habituation during repeated exposure to fearful and neutral faces: A functional mri study. Brain Research Bulletin, 59, 387-392

Fox, M. D., & Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci, 8, 700-711

Fransson, P. (2006). How default is the default mode of brain function? Further evidence from intrinsic bold signal fluctuations. Neuropsychologia, 44, 2836-2845

Friston, K. J., Harrison, L., & Penny, W. (2003). Dynamic causal modelling. Neuroimage, 19, 1273-1302

Fu, C. H., Williams, S. C., Cleare, A. J., Scott, J., Mitterschiffthaler, M. T., Walsh, N. D., Donaldson, C., Suckling, J., Andrew, C., Steiner, H., & Murray, R. M. (2008). Neural responses to sad facial expressions in major depression following cognitive behavioral therapy. Biol Psychiatry,

Fusar-Poli, P., Placentino, A., Carletti, F., Landi, P., Allen, P., Surguladze, S., Benedetti, F., Abbamonte, M., Gasparotti, R., Barale, F., Perez, J., Mcguire, P., & Politi, P. (2009). Functional atlas of emotional faces processing: A voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. J Psychiatry Neurosci, 34, 418-432

Gaffrey, M. S., Luby, J. L., Botteron, K., Repovs, G., & Barch, D. M. (2012). Default mode network connectivity in children with a history of preschool onset depression. J Child Psychol Psychiatry,

Garrett, A. S., Carrion, V., Kletter, H., Karchemskiy, A., Weems, C. F., & Reiss, A. (2012). Brain activation to facial expressions in youth with ptsd symptoms. Depress Anxiety, 29, 449-459

Gee, D. G., Gabard-Durnam, L. J., Flannery, J., Goff, B., Humphreys, K. L., Telzer, E. H., Hare, T. A., Bookheimer, S. Y., & Tottenham, N. (2013a). Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. Proc. Natl. Acad. Sci. U.S.A., 110, 15638-15643

Gee, D. G., Humphreys, K. L., Flannery, J., Goff, B., Telzer, E. H., Shapiro, M., Hare, T. A., Bookheimer, S. Y., & Tottenham, N. (2013b). A developmental shift from positive to negative connectivity in human amygdala-prefrontal circuitry. J. Neurosci., 33, 4584-4593

Giedd, J. N., Blumenthal, J., Jeffries, N. O., Castellanos, F. X., Liu, H., Zijdenbos, A., Paus, T., Evans, A. C., & Rapoport, J. L. (1999). Brain development during childhood and adolescence: A longitudinal mri study. Nat. Neurosci., 2, 861-863

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., Nugent, T. F. R., Herman, D. H., Clasen, L. S., Toga, A. W., Rapoport, J. L., & Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. Proc. Natl. Acad. Sci. U.S.A., 101, 8174-8179

Grecucci, A., Giorgetta, C., Bonini, N., & Sanfey, A. G. (2013). Reappraising social emotions: The role of inferior frontal gyrus, temporo-parietal junction and insula in interpersonal emotion regulation. Front Hum Neurosci, 7, 523

Grossmann, T., & Johnson, M. H. (2007). The development of the social brain in human infancy. Eur J Neurosci, 25, 909-919

Guyer, A. E., Monk, C. S., Clure-Tone, E. B., Nelson, E. E., Roberson-Nay, R., Adler, A. D., Fromm, S., Leibenluft, E., Pine, D. S., & Ernst, M. (2008). A developmental examination of amygdala response to facial expressions. Journal of Cognitive Neuroscience,

Hare, T. A., Tottenham, N., Galvan, A., Voss, H. U., Glover, G. H., & Casey, B. J. (2008). Biological substrates of emotional reactivity and regulation in adolescence during an emotional go-nogo task. Biol Psychiatry, 63, 927-934

Hart, H., & Rubia, K. (2012). Neuroimaging of child abuse: A critical review. Front Hum Neurosci, 6, 52

Hulvershorn, L. A., Cullen, K., & Anand, A. (2011). Toward dysfunctional connectivity: A review of neuroimaging findings in pediatric major depressive disorder. Brain Imaging and Behavior, 5, 307-328

In-Albon, T., & Schneider, S. (2007). Psychotherapy of childhood anxiety disorders: A meta analysis. Psychother Psychosom, 76, 15-24

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., Sanislow, C., & Wang, P. (2010). Research domain criteria (rdoc): Toward a new classification framework for research on mental disorders. Am J Psychiatry, 167, 748-751

Jenkinson, M., Bannister, P., Brady, M., & Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage, 17, 825-841

Jiao, Q., Ding, J., Lu, G., Su, L., Zhang, Z., Wang, Z., Zhong, Y., Li, K., Ding, M., & Liu, Y. (2011). Increased activity imbalance in fronto-subcortical circuits in adolescents with major depression. Plos One, 6, e25159

Jin, C., Gao, C., Chen, C., Ma, S., Netra, R., Wang, Y., Zhang, M., & Li, D. (2011). A preliminary study of the dysregulation of the resting networks in first-episode medication-naive adolescent depression. Neurosci Lett, 503, 105-109

Johnstone, T., Somerville, L. H., Alexander, A. L., Oakes, T. R., Davidson, R. J., Kalin, N. H., & Whalen, P. J. (2005). Stability of amygdala bold response to fearful faces over multiple scan sessions. Neuroimage, 25, 1112-1123

Johnstone, T., Van Reekum, C. M., Urry, H. L., Kalin, N. H., & Davidson, R. J. (2007). Failure to regulate: Counterproductive recruitment of top-down prefrontal-subcortical circuitry in major depression. J Neurosci, 27, 8877-8884

Kessler, R. C., Avenevoli, S., Costello, E. J., Georgiades, K., Green, J. G., Gruber, M. J., He, J.-P., Koretz, D., Mclaughlin, K. A., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Merikangas, K. R. (2012a). Prevalence, persistence, and sociodemographic correlates of dsm-iv disorders in the national comorbidity survey replication adolescent supplement. Arch. Gen. Psychiatry, 69, 372-380

Kessler, R. C., Avenevoli, S., Costello, J., Green, J. G., Gruber, M. J., Mclaughlin, K. A., Petukhova, M., Sampson, N. A., Zaslavsky, A. M., & Merikangas, K. R. (2012b). Severity of 12-month dsm-iv disorders in the national comorbidity survey replication adolescent supplement. Arch Gen Psychiatry, 69, 381-389

Kotov, R., Gamez, W., Schmidt, F., & Watson, D. (2010). Linking "big" personality traits to anxiety, depressive, and substance use disorders: A meta-analysis. Psychol Bull, 136, 768-821

Lewinsohn, P. M., Gotlib, I. H., & Seeley, J. R. (1995). Adolescent psychopathology: Iv. Specificity of psychosocial risk factors for depression and substance abuse in older adolescents. J Am Acad Child Adolesc Psychiatry, 34, 1221-1229

Lindert, J., Von Ehrenstein, O. S., Grashow, R., Gal, G., Braehler, E., & Weisskopf, M. G. (2014). Sexual and physical abuse in childhood is associated with depression and anxiety over the life course: Systematic review and meta-analysis. International Journal of Public Health, 59, 359-372

Månsson, K. N. T., Carlbring, P., Frick, A., Engman, J., Olsson, C.-J., Bodlund, O., Furmark, T., & Andersson, G. (2013). Altered neural correlates of affective processing after internet-delivered cognitive behavior therapy for social anxiety disorder. Psychiatry Research: Neuroimaging, 214, 229-237

Maslowsky, J., Mogg, K., Bradley, B. P., Mcclure-Tone, E., Ernst, M., Pine, D. S., & Monk, C. S. (2010). A preliminary investigation of neural correlates of treatment in adolescents with generalized anxiety disorder. J Child Adolesc Psychopharmacol, 20, 105-111

Mayberg, H. S. (1997). Limbic-cortical dysregulation: A proposed model of depression. Journal of Neuropsychiatry and Clinical Neurosciences, 9, 471-481

Mcclure, E. B., Adler, A., Monk, C. S., Cameron, J., Smith, S., Nelson, E. E., Leibenluft, E., Ernst, M., & Pine, D. S. (2007a). Fmri predictors of treatment outcome in pediatric anxiety disorders. Psychopharmacology (Berl), 191, 97-105

Mcclure, E. B., Monk, C. S., Nelson, E. E., Parrish, J. M., Adler, A., Blair, R. J. R., Fromm, S., Charney, D. S., Leibenluft, E., Ernst, M., & Pine, D. S. (2007b). Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder. Arch. Gen. Psychiatry, 64, 97-106

Merikangas, K. R., He, J. P., Burstein, M., Swanson, S. A., Avenevoli, S., Cui, L., Benjet, C., Georgiades, K., & Swendsen, J. (2010). Lifetime prevalence of mental disorders in u.S. Adolescents: Results from the national comorbidity survey replication--adolescent supplement (ncs-a). J Am Acad Child Adolesc Psychiatry, 49, 980-989

Mingtian, Z., Shuqiao, Y., Xiongzhao, Z., Jinyao, Y., Xueling, Z., Xiang, W., Yingzi, L., Jian,\ L., & Wei, W. (2012). Elevated amygdala activity to negative faces in young adults with early onset major depressive disorder. Psychiatry Res, 201, 107-112

Monk, C. S., Grillon, C., Baas, J. M., Mcclure, E. B., Nelson, E. E., Zarahn, E., Charney, D. S., Ernst, M., & Pine, D. S. (2003a). A neuroimaging method for the study of threat in adolescents. Dev.Psychobiol., 43, 359-366

Monk, C. S., Mcclure, E. B., Nelson, E. E., Zarahn, E., Bilder, R. M., Leibenluft, E., Charney, D. S., Ernst, M., & Pine, D. S. (2003b). Adolescent immaturity in attention-related brain engagement to emotional facial expressions. Neuroimage, 20, 420-428

Monk, C. S. (2008). The development of emotion-related neural circuitry in health and psychopathology. Dev Psychopathol, 20, 1231-1250

Monk, C. S., Klein, R. G., Telzer, E. H., Schroth, E. A., Mannuzza, S., Moulton, J. L., Guardino, M., Masten, C. L., Mcclure-Tone, E. B., Fromm, S., Blair, R. J., Pine, D. S., & Ernst, M. (2008a). Amygdala and nucleus accumbens activation to emotional facial expressions in children and adolescents at risk for major depression. Am J Psychiatry, 165, 90-98

Monk, C. S., Telzer, E. H., Mogg, K., Bradley, B. P., Mai, X., Louro, H. M., Chen, G., Clure Tone, E. B., Ernst, M., & Pine, D. S. (2008b). Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. Arch. Gen. Psychiatry, 65, 568-576

Moore, W. E., 3rd, Pfeifer, J. H., Masten, C. L., Mazziotta, J. C., Iacoboni, M., & Dapretto, M. (2012). Facing puberty: Associations between pubertal development and neural responses to affective facial displays. Soc Cogn Affect Neurosci, 7, 35-43

Pannekoek, J. N., Van Der Werff, S. J., Meens, P. H., Van Den Bulk, B. G., Jolles, D. D., Veer, I. M., Van Lang, N. D., Rombouts, S. A., Van Der Wee, N. J., & Vermeiren, R. R. (2014a). Aberrant resting-state functional connectivity in limbic and salience networks in treatment-naive clinically depressed adolescents. J Child Psychol Psychiatry,

Pannekoek, J. N., Van Der Werff, S. J., Van Den Bulk, B. G., Van Lang, N. D., Rombouts, S. A., Van Buchem, M. A., Vermeiren, R. R., & Van Der Wee, N. J. (2014b). Reduced anterior cingulate gray matter volume in treatment-naive clinically depressed adolescents. Neuroimage Clin, 4, 336-342

Pattwell, S. S., Duhoux, S., Hartley, C. A., Johnson, D. C., Jing, D., Elliott, M. D., Ruberry, E. J., Powers, A., Mehta, N., Yang, R. R., Soliman, F., Glatt, C. E., Casey, B. J., Ninan, I., & Lee, F. S. (2012). Altered fear learning across development in both mouse and human. Proc Natl Acad Sci U S A, 109, 16318-16323

Paus, T. (2005). Mapping brain maturation and cognitive development during adolescence. Trends Cogn Sci, 9, 60-68

Perlman, G., Simmons, A. N., Wu, J., Hahn, K. S., Tapert, S. F., Max, J. E., Paulus, M. P., Brown, G. G., Frank, G. K., Campbell-Sills, L., & Yang, T. T. (2012). Amygdala response and functional connectivity during emotion regulation: A study of 14 depressed adolescents. J Affect Disord, 139, 75-84

Pfeifer, J. H., Masten, C. L., Moore, W. E. R., Oswald, T. M., Mazziotta, J. C., Iacoboni, M., & Dapretto, M. (2011). Entering adolescence: Resistance to peer influence, risky behavior, and neural changes in emotion reactivity. Neuron, 69, 1029-1036

Phelps, E. A., & Ledoux, J. E. (2005). Contributions of the amygdala to emotion processing: From animal models to human behavior. Neuron, 48, 175-187

Pine, D. S., Cohen, P., Gurley, D., Brook, J., & Ma, Y. (1998). The risk for early-adulthood anxiety and depressive disorders in adolescents with anxiety and depressive disorders. Arch. Gen. Psychiatry, 55, 56-64

Pine, D. S. (2007). Research review: A neuroscience framework for pediatric anxiety disorders. J Child Psychol Psychiatry, 48, 631-648

Pine, D. S., Guyer, A. E., & Leibenluft, E. (2008). Functional magnetic resonance imaging and pediatric anxiety. J Am Acad Child Adolesc Psychiatry, 47, 1217-1221

Plichta, M. M., Schwarz, A. J., Grimm, O., Morgen, K., Mier, D., Sauer, C., Gerdes, A. B., Haddad, L., Tost, H., Esslinger, C., Colman, P., Wilson, F., Kirsch, P., & Meyer-Lindenberg, A. (2012). Test-retest reliability of evoked bold signals from a cognitive-emotive fmri test battery. Neuroimage,

Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity mri networks arise from subject motion. Neuroimage, 59, 2142-2154

Quide, Y., Witteveen, A. B., El-Hage, W., Veltman, D. J., & Olff, M. (2012). Differences between effects of psychological versus pharmacological treatments on functional and morphological brain alterations in anxiety disorders and major depressive disorder: A systematic review. Neurosci Biobehav Rev, 36, 626-644

Roberson-Nay, R., Mcclure, E. B., Monk, C. S., Nelson, E. E., Guyer, A. E., Fromm, S. J., Charney, D. S., Leibenluft, E., Blair, J., Ernst, M., & Pine, D. S. (2006). Increased amygdala activity during successful memory encoding in adolescent major depressive disorder: An fmri study. Biol Psychiatry, 60, 966-973

Roy, A. K., Shehzad, Z., Margulies, D. S., Kelly, A. M., Uddin, L. Q., Gotimer, K., Biswal, B. B., Castellanos, F. X., & Milham, M. P. (2009). Functional connectivity of the human amygdala using resting state fmri. Neuroimage, 45, 614-626

Roy, A. K., Fudge, J. L., Kelly, C., Perry, J. S., Daniele, T., Carlisi, C., Benson, B., Castellanos, F. X., Milham, M. P., Pine, D. S., & Ernst, M. (2013). Intrinsic functional connectivity of amygdala-based networks in adolescent generalized anxiety disorder. J Am Acad Child Adolesc Psychiatry, 52, 290-299.e292

Saad, Z. S., Gotts, S. J., Murphy, K., Chen, G., Jo, H. J., Martin, A., & Cox, R. W. (2012). Trouble at rest: How correlation patterns and group differences become distorted after global signal regression. Brain connectivity, 2, 25-32

Satterthwaite, T. D., Elliott, M. A., Gerraty, R. T., Ruparel, K., Loughead, J., Calkins, M. E., Eickhoff, S. B., Hakonarson, H., Gur, R. C., Gur, R. E., & Wolf, D. H. (2013). An improved framework for confound regression and filtering for control of motion artifact in the preprocessing of resting-state functional connectivity data. Neuroimage, 64, 240-256

Sauer, A., Mothes-Lasch, M., Miltner, W. H., & Straube, T. (2013). Effects of gaze direction, head orientation and valence of facial expression on amygdala activity. Soc Cogn Affect Neurosci,

Scherf, K. S., Luna, B., Avidan, G., & Behrmann, M. (2011). “what” precedes “which”: Developmental neural tuning in face- and place-related cortex. Cerebral Cortex,

Scherf, K. S., Behrmann, M., & Dahl, R. E. (2012). Facing changes and changing faces in adolescence: A new model for investigating adolescent-specific interactions between pubertal, brain and behavioral development. Dev Cogn Neurosci, 2, 199-219

Scherf, K. S., Smyth, J. M., & Delgado, M. R. (2013). The amygdala: An agent of change in adolescent neural networks. Horm Behav, 64, 298-313

Schwartz, C. E., Wright, C. I., Shin, L. M., Kagan, J., Whalen, P. J., Mcmullin, K. G., & Rauch, S. L. (2003). Differential amygdalar response to novel versus newly familiar neutral faces: A functional mri probe developed for studying inhibited temperament. Biol Psychiatry, 53, 854-862

Shaw, D. J., Grosbras, M. H., Leonard, G., Pike, G. B., & Paus, T. (2011). Development of functional connectivity during adolescence: A longitudinal study using an action-observation paradigm. Journal of Cognitive Neuroscience, 23, 3713-3724

Shaw, D. J., Grosbras, M. H., Leonard, G., Pike, G. B., & Paus, T. (2012). Development of the action observation network during early adolescence: A longitudinal study. Soc Cogn Affect Neurosci, 7, 64-80

Shechner, T., Britton, J. C., Perez-Edgar, K., Bar-Haim, Y., Ernst, M., Fox, N. A., Leibenluft, E., & Pine, D. S. (2012). Attention biases, anxiety, and development: Toward or away from threats or rewards? Depress Anxiety,

Siegle, G. J., Carter, C. S., & Thase, M. E. (2006). Use of fmri to predict recovery from unipolar depression with cognitive behavior therapy. Am J Psychiatry, 163, 735-738

Simms, L. J., Prisciandaro, J. J., Krueger, R. F., & Goldberg, D. P. (2012). The structure of depression, anxiety and somatic symptoms in primary care. Psychol Med, 42, 15-28

Sladky, R., Hoeflich, A., Atanelov, J., Kraus, C., Baldinger, P., Moser, E., Lanzenberger, R., & Windischberger, C. (2012). Increased neural habituation in the amygdala and orbitofrontal cortex in social anxiety disorder revealed by fmri. Plos One, 7,

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E., Johansen Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural mr image analysis and implementation as fsl. Neuroimage, 23 Suppl 1, S208-219

Somerville, L. H., Kim, H., Johnstone, T., Alexander, A. L., & Whalen, P. J. (2004). Human amygdala responses during presentation of happy and neutral faces: Correlations with state anxiety. Biol Psychiatry, 55, 897-903

Somerville, L. H., & Casey, B. J. (2010). Developmental neurobiology of cognitive control and motivational systems. Curr Opin Neurobiol, 20, 236-241

Somerville, L. H., Fani, N., & Clure-Tone, E. B. (2011). Behavioral and neural representation of emotional facial expressions across the lifespan. Dev.Neuropsychol., 36, 408-428

Stein, M. B., Simmons, A. N., Feinstein, J. S., & Paulus, M. P. (2007). Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. Am J Psychiatry, 164, 318-327

Steinberg, L. (2005). Cognitive and affective development in adolescence. Trends Cogn. Sci., 9, 69-74

Strawn, J. R., Bitter, S. M., Weber, W. A., Chu, W.-J., Whitsel, R. M., Adler, C., Cerullo, M. A., Eliassen, J., Strakowski, S. M., & Delbello, M. P. (2012a). Neurocircuitry of generalized anxiety disorder in adolescents: A pilot functional neuroimaging and functional connectivity study. Depress Anxiety, 29, 939-947

Strawn, J. R., Wehry, A. M., Delbello, M. P., Rynn, M. A., & Strakowski, S. (2012b). Establishing the neurobiologic basis of treatment in children and adolescents with generalized anxiety disorder. Depress Anxiety, 29, 328-339

Tahmasebi, A. M., Artiges, E., Banaschewski, T., Barker, G. J., Bruehl, R., Buchel, C., Conrod, P. J., Flor, H., Garavan, H., Gallinat, J., Heinz, A., Ittermann, B., Loth, E., Mareckova, K., Martinot, J. L., Poline, J. B., Rietschel, M., Smolka, M. N., Strohle, A., Schumann, G., & Paus, T. (2012). Creating probabilistic maps of the face network in the adolescent brain: A multicentre functional mri study. Hum. Brain Mapp, 33, 938-957

Thapar, A., Collishaw, S., Pine, D. S., & Thapar, A. K. (2012). Depression in adolescence. Lancet,

Thomas, K. M., Drevets, W. C., Dahl, R. E., Ryan, N. D., Birmaher, B., Eccard, C. H., Axelson, D., Whalen, P. J., & Casey, B. J. (2001a). Amygdala response to fearful faces in anxious and depressed children. Arch. Gen. Psychiatry, 58, 1057-1063

Thomas, K. M., Drevets, W. C., Whalen, P. J., Eccard, C. H., Dahl, R. E., Ryan, N. D., Casey, B. J. (2001b). Amygdala response to facial expressions in children and adults. Biol Psychiatry, 49, 309-316

Toro, R., Fox, P. T., & Paus, T. (2008). Functional coactivation map of the human brain. Cereb Cortex, 18, 2553-2559

Tottenham, N., Hare, T., & Casey, B. (2009a). A developmental perspective on human amygdala function in The human amygdala. Guilford Press: New York, NY

Tottenham, N., Tanaka, J. W., Leon, A. C., Mccarry, T., Nurse, M., Hare, T. A., Marcus, D. J., Westerlund, A., Casey, B. J., & Nelson, C. (2009b). The nimstim set of facial expressions: Judgments from untrained research participants. Psychiatry Res, 168, 242-249

Van Den Bulk, B. G., Koolschijn, P. C., Meens, P. H., Van Lang, N. D., Van Der Wee, N. J., Rombouts, S. A., Vermeiren, R. R., & Crone, E. A. (2013). How stable is activation in the amygdala and prefrontal cortex in adolescence? A study of emotional face processing across three measurements. Dev Cogn Neurosci, 4, 65-76

Van Den Bulk, B. G., Meens, P. H., Van Lang, N. D., De Voogd, E. L., Van Der Wee, N. J., Rombouts, S. A., Crone, E. A., & Vermeiren, R. R. (2014). Amygdala activation during emotional face processing in adolescents with affective disorders: The role of underlying depression and anxiety symptoms. Front Hum Neurosci, 8, 393

Van Harmelen, A. L., Van Tol, M. J., Demenescu, L. R., Van Der Wee, N. J., Veltman, D. J., Aleman, A., Van Buchem, M. A., Spinhoven, P., Penninx, B. W., & Elzinga, B. M. (2013). Enhanced amygdala reactivity to emotional faces in adults reporting childhood emotional maltreatment. Soc Cogn Affect Neurosci, 8, 362-369

Veer, I. M., Oei, N. Y., Spinhoven, P., Van Buchem, M. A., Elzinga, B. M., & Rombouts, S. A. (2011). Beyond acute social stress: Increased functional connectivity between amygdala and cortical midline structures. Neuroimage, 57, 1534-1541

Walkup, J. T., Albano, A. M., Piacentini, J., Birmaher, B., Compton, S. N., Sherrill, J. T., Ginsburg, G. S., Rynn, M. A., Mccracken, J., Waslick, B., Iyengar, S., March, J. S., & Kendall, P. C. (2008). Cognitive behavioral therapy, sertraline, or a combination in childhood anxiety. N Engl J Med, 359, 2753-2766

Weissenbacher, A., Kasess, C., Gerstl, F., Lanzenberger, R., Moser, E., & Windischberger, C. (2009). Correlations and anticorrelations in resting-state functional connectivity mri: A quantitative comparison of preprocessing strategies. Neuroimage, 47, 1408-1416

Wendt, J., Schmidt, L. E., Lotze, M., & Hamm, A. O. (2012). Mechanisms of change: Effects of repetitive exposure to feared stimuli on the brain's fear network. Psychophysiology, 49, 1319-1329

Whalen, P. J. (1998). Fear, vigilance, and ambiguity: Initial neuroimaging studies of the human amygdala. Current Directions in Psychological Science, 7, 177-188

Whalen, P. J., Davis, F. C., Oler, J. A., Kim, H., Kim, M., & Neta, M. (2009). Human amygdala response to facial expressions of emotion in The human amygdala. The guilford Press: New York, NY

Williams, L. M., Brown, K. J., Palmer, D., Liddell, B. J., Kemp, A. H., Olivieri, G., Peduto, A., & Gordon, E. (2006). The mellow years?: Neural basis of improving emotional stability over age. J. Neurosci., 26, 6422-6430

Yang, T. T., Simmons, A. N., Matthews, S. C., Tapert, S. F., Frank, G. K., Max, J. E., Bischoff-Grethe, A., Lansing, A. E., Brown, G., Strigo, I. A., Wu, J., & Paulus, M. P. (2010). Adolescents with major depression demonstrate increased amygdala activation. Journal of the American Academy of Child & Adolescent Psychiatry, 49, 42-51

Zisook, S., Lesser, I., Stewart, J. W., Wisniewski, S. R., Balasubramani, G. K., Fava, M., & Gilmer, W. S. (2007). Effect of age at onset on the course of major depressive disorder. American Journal of Psychiatry, 164, 1539-1546

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Curriculum Vitae

Bianca Gabriëlla van den Bulk was born in Leidschendam, the Netherlands, on October 16, 1985. She obtained her Gymnasium degree from Melanchthon College Schiebroek in 2004. In the same year she started to study psychology at Leiden University. She obtained her research master degree in developmental psychology in the summer of 2009. As part of her research master internship and thesis Bianca was involved in a neuroimaging study on developmental changes in feedback learning under supervision of Dr. Wouter van den Bos and Prof. Dr. Eveline Crone. After graduating, Bianca worked as a research assistant at the department of child and adolescents psychiatry of the Leiden University Medical Center, Curium-LUMC. Meanwhile, she also did an internship within the clinical practice and she completed several clinically oriented courses within the master of developmental psychology at Leiden University. In February 2011, Bianca started as a PhD.-student within the larger EPISCA project at Curium-LUMC under supervision of Prof. Dr. Robert Vermeiren (Curium-LUMC) and Prof. Dr. Eveline Crone (Brain and Development Lab, Leiden University). Her research focussed on the neurobiological mechanisms underlying adolescent onset depression and anxiety disorders. During her Ph.D. trajectory she visited Dr. Leah Somerville of the Affective Neuroscience and Development Lab at Harvard University, MA USA. In August 2014, Bianca started as a postdoctoral researcher within the Leiden Consortium on Individual Development at the department of Child and Family Studies at Leiden University under supervision of Prof. Dr. Rien van IJzendoorn and Prof. Dr. Marian Bakermans-Kranenburg.

List of publications

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van den Bulk, B.G., Meens, P.H.F., van Lang, N.D.J., de Voogd, E.L., van der Wee, N.J.A., Rombouts, S.A.R.B., Crone, E.A., Vermeiren, R.R.J.M. (2014). Amygdala activation during emotional faces processing in adolescents with affective disorders: the role of underlying depres sion and anxiety symptoms. Frontiers in Human Neuroscience, 8, #393.

Pannekoek, J.N., van der Werff, S.J.A., Meens, P.H.F., **van den Bulk, B.G.**, Jolles, D.D., Veerm I.M., van Lang, N.D.J., Rombouts, S.A.R.B., van der Wee, N.J.A. & Vermeiren, R.R.J.M. (2014). Aberrant resting-state functional connectivity in limbic and salience networks in treatment-naïve clinically depressed adolescents. Journal of Child Psychology & Psychiatry, doi: 10.1111/jcpp.12266.

Aghajani, M., Veer, I.M., van Lang, N.D.J., Meens, P.H.F., **van den Bulk, B.G.**, Rombouts, S.A.R.B., Vermeiren, R.R.J.M. & van der Wee (2014). Alte red white-matter architecture in treatment-naive adolescents with clinical depression. Psychological Medicine, doi:10.1017/S0033291713003000

Pannekoek, J.N., van der Werff, S.J.A., **van den Bulk, B.G.**, van Lang, N.D.J., Rombouts, S.A.R.B., van Buchem, M.A., Vermeiren, R.R.J.M. & van der Wee, N.J.A. (2014). Reduced anterior cingulate gray matter volume in treatment-naive clinically depressed adolescents. NeuroImage: Clinical, 4, 336-342.

Somerville, L.H., **van den Bulk, B.G.** & Skwara, A.C. (2014). Response to: The triadic model perspective for the study of adolescent motivated behavior. Brain and Cognition, http://dx.doi.org/10.1016/j.bandc.2014.01.003

van den Bulk, B.G., Koolschijn, P.C.M.P, Meens, P.H.F., Lang, N.D.J., van der Wee, N.J.A., Rombouts, S.A.R.B., Vermeiren, R.R.J.M. & Crone, E.A. (2013). How stable is activation in the amygdala and prefrontal cortex in adolescence? A study of emotional face processing across three measurements. Developmental Cognitive Neuroscience, 4, 65-74.

van den Bos, W., Güroğlu, B., **van den Bulk, B.G.**, Rombouts, S.A.R.B. & Crone, E.A.M. (2009). Better than expected or as bad as you thought? The neurocognitive development of probabilistic feedback processing. Frontiers in Human Neuroscience, 3 (52), 1-11.

Submitted

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.

van den Bulk, B.G., Somerville, L.H., van Hoof, M.J., van Lang, N.D.J., van der Wee, N.J.A., Crone, E.A., Vermeiren, R.R.J.M. Habituation effects during emotional face processing in adolescents with internalizing disorders, sexually abused adolescents and healthy controls.

van den Bulk, B.G., van der Werff, S.J.A., Aghajani, M., Granettia, M., van Lang, N.D.J., van der Wee, N.J.A., Rombouts, S.A.R.B., Crone, E.A., Vermeiren, R.R.J.M. Longitudinal changes in resting-state functional connectivity in depressed and anxious adolescents in relation to treatment.

Dutch publications

van den Bulk., B.G. Hoe stabiel en betrouwbaar is hersenactiviteit in de amygdala en prefrontale cortex? Een longitudinale studie bij gezonde adolescenten. Neuropraxis, 2, 56-62.