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

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## 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 the 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; Quidé 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; Wellcome 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 extend 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.

