Mood related insights : functional and structural MRI studies in depression and anxiety disorders

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CHAPTER 8

SUMMARY
GENERAL DISCUSSION
FUTURE PERSPECTIVES

Brain structure and brain function in patients with Major Depressive Disorder (MDD) and prevalent anxiety disorders were studied in this thesis. The first aim of this study was to identify disorder specific and shared neuroanatomical and functional abnormalities, with a focus on emotional information processing and executive functions. Structural and functional MRI patterns of patients with both MDD and anxiety disorders (comorbid depression-anxiety) were directly compared with those of patients with only MDD or anxiety disorders. The second aim of this study was to study the relation of common risk factors of mood- and anxiety disorders, namely neuroticism and childhood emotional abuse, and regional brain volume. Giving insight into the shared and distinct neurobiological underpinnings of MDD and anxiety disorders is important to understand their high comorbidity, for the classification of MDD and anxiety disorders, and subsequently for the treatment and course of these highly prevalent psychiatric disorders.

Both brain morphometry and brain function related to cognitive and emotional processes were investigated with the use of magnetic resonance imaging (MRI). The anxiety disorders included in this study were social anxiety disorder, panic disorder, and generalized anxiety disorder. The studies described in this thesis are based on data acquired in the context of the Netherlands Study of Depression and Anxiety (NESDA), a large scale, multi-site, observational cohort study. In this final chapter, we will summarize and discuss the main results of the studies presented (Chapter 2-7). Finally, implications and recommendations for future research are provided.

SUMMARY OF RESULTS

The question whether MDD, comorbid depression-anxiety disorder, and anxiety disorders were associated with volumetric abnormalities in regions linked to emotion perception and regulation was adressed first. In **Chapter 2**, we showed that reduced volume of the rostral and dorsal anterior cingulate cortex (ACC) is a generic effect in MDD and anxiety disorders. This ACC reduction was observed independent of comorbidity and was unrelated to variations in illness severity, medication use, and sex. This generic effect supports the notion of a shared aetiology in MDD and anxiety disorders and may reflect a common symptom dimension related to altered emotion processing. Decreased regional volume of the inferior frontal cortex in MDD and lateral temporal cortex in anxiety disorders without comorbid MDD, on the other hand, may reflect disorder-specific symptom clusters. In addition, early onset of depression was found to be associated with reduced volume of the subgenual ACC, extending into the orbitofrontal gyrus, compared with healthy controls. This additional volume reduction in the affective subdivision of the ACC further suggests that vulnerability for MDD is related to volume of the ventral ACC.

The neural (functional MRI) correlates of emotional word memory in MDD and anxiety disorders were investigated in the next chapter. Studying neural

correlates of emotional memory processes is of interest to MDD and anxiety disorders, because abnormalities in the encoding and remembering of emotional information might reinforce negative mood and could contribute to the prolonged course of the disease (Elliott et al., 2002). In **Chapter 3**, we demonstrated that decreased activation of the hippocampus during positive word encoding is a common phenomenon in depression and anxiety disorders that may constitute a common vulnerability for biased information processing in MDD and anxiety disorders. This effect was observed independent of illness severity, medication use, and regional volume. Moderately and severely depressed MDD patients were additionally characterized by increased activation of the insula, amygdala, and by trend-wise increased activation of the striatum and prefrontal cortex during encoding of negative words. These findings may mark an increased sensitivity for negative stimuli. Furthermore, illness severity dependent increased activation of fronto-polar regions in comorbid depression-anxiety, irrespective of valence was observed. These results emphasize the current distinction between MDD and anxiety disorders (with and without comorbid MDD) with respect to processing of mood-congruent information (i.e., negative words), although a hippocampal hypo-response to positive words may mark a general insensitiveness to positive information.

The involvement of dorsal prefrontal regions such as the dorsal ACC, dorsolateral PFC, and dorsomedial PFC during the non-emotional Tower of London visuospatial planning task was studied in **Chapter 4.** Here, we demonstrated that only moderately to severely depressed MDD patients are characterized by increased left dorsolateral prefrontal activity as a function of executive task load together with subtle performance differences. Mildly depressed and remitted MDD patients, as well as anxiety patients showed normal task performance and no activation differences were observed. Combined these results indicate that prefrontal hyperactivation during high planning demands is a state (i.e., depressive episode dependent) characteristic and not a trait (i.e., depressive episode independent) characteristic of MDD. We conclude that executive dysfunction during a non-emotional visuospatial planning task is not a feature of anxiety disorders, supporting the current diagnostic distinction between anxiety disorders and depression.

In **Chapter 5**, we studied whole-brain connectivity during an emotional word classification task using template independent component networks. We aimed to directly investigate the connectivity of subcortical and (para)limbic regions associated with emotion perception and generation of emotional states on the one hand, and dorsal cortical regions associated with emotional regulation on the other hand. We demonstrated that patients with MDD who were life-time free of anxiety disorders showed decreased connectivity of the medial PFC and ventral-striatal regions within the salience network (Seeley et al., 2007) during the classification of negative, neutral, and positive words, unrelated to illness severity. Furthermore, connectivity of the medial PFC with the task-executive

network was positively related to the number of words classified as positive in the MDD group. This decreased connectivity of fronto-striatal regions within the salience compared with controls confirms the hypothesis of a relative ventral-dorsal decoupling that may serve to explain the compromised emotional regulation in MDD.

In **Chapter 6** we focused on the long-term anatomical correlates of childhood emotional maltreatment. We demonstrated that childhood emotional maltreatment is associated with lower volume of the dorsal medial PFC, independent of current psychopathological status and independent of concomitant physical or sexual abuse. This finding suggests that sustained inhibition of growth or structural damage can occur after exposure to childhood emotional maltreatment. Given the important role of the dorsal medial prefrontal cortex in the regulation of emotional behavior, our results may provide an important link in understanding the increased emotional sensitivity in individuals reporting childhood emotional maltreatment.

The correlations of the personality traits neuroticism and extraversion was studied in **Chapter 7**. We showed that orbitofrontal and subgenual ACC volume in healthy control participants was positively related to extraversion. No relation with neuroticism was observed. In addition, extraversion was positively correlated to right amygdalar volume. These findings indicate that extraversion is a potent factor in predicting regional brain volume in structures related to emotional processing. High levels of extraversion may modulate emotion processing through the orbitofrontal cortex, thereby affecting the likelihood of developing affective disorders.

Taken together, these findings suggest that MDD and common anxiety disorders share neuroanatomical and neurophysiological abnormalities, but at the same time are characterized by unique neuroanatomical abnormalities and activation patterns during cognitive and emotional processes. Next, we showed that besides current psychopathology, personality factors and emotional traumatic experiences during childhood are associated with variations in volume of structures that are important for mood regulation. Therefore, these effects could mediate the vulnerability for developing MDD and anxiety disorders. Overall, no effect of SSRI use on functional and structural measures was observed.

This is the first series of neuroimaging studies that studied MDD, prevalent anxiety disorders, and comorbid depression-anxiety together, while at the same time controlled for important factors such as medication use and illness severity.

In this section, the shared neuroanatomical- and functional MRI correlates of MDD and anxiety disorders will be discussed, followed by a discussion of the unique neurophysiological correlates of MDD and anxiety disorders. Next, the question of whether to regard comorbid depression-anxiety as MDD plus anxiety or as a different diagnostic category will be discussed. Subsequently, risk factors of mood and anxiety disorders will be discussed. Also, the result of relatively normal cognitive functioning in our sample will receive attention. Furthermore, the stability of effects and the question of causality will be addressed. Finally, the present results will be reflected on in light of the frequently used neuroanatomical models of mood regulation and depressive pathology. Considerations and implications of the results presented in this thesis will be discussed.

MDD = ANXIETY DISORDERS

This study demonstrated for the first time that MDD and anxiety disorders share morphologic and activation abnormalities. Both MDD patients and patients with anxiety disorders showed reduced volume of the dorsal and rostral ACC. This volumetric abnormality was also observed in patients with both MDD and anxiety disorders. ACC reductions have been previously observed in MDD and panic disorder, but groups had not been compared directly. Also, studies in MDD and anxiety disorders performed to date were often confounded by the inclusion of comorbid anxiety or MDD, respectively, and the specificity of these findings remained therefore unclear. Evidence is now provided that suggests that MDD and anxiety disorders share a common neuroanatomical characteristic that may explain, at least in part, the emotional dysregulation that is associated with both anxiety disorders and MDD. Interestingly, this abnormality is observed in the rostral ACC, a region that has been considered the emotion-cognition integration component in the Mayberg model (1997) (See **chapter 1**). A common abnormality in this region in both depression and anxiety indicates that regulation between dorsal and lateral cortical regulatory and subcortical and (para)limbic appraisal regions may be compromised owing to this structural abnormality. This rostral ACC abnormality may lead to sub optimal frontal-subcortical regulation, resulting in a variety of symptoms associated with both mood and anxiety disorders.

Next, a common abnormality in MDD and anxiety disorders was observed during the encoding of mood-incongruent (i.e., positive) words. MDD and anxiety patients showed less activation of the right hippocampus, also when a diagnosis of both depression and anxiety was present. This suggests that abnormal processing of positive information might be considered a trait phenomenon of both depression and anxiety disorders, because the effect was observed independent of illness severity. This suggestion is further supported by the word classification pattern that was observed in both MDD and anxiety

disorders: fewer words were classified as positive compared with controls, whereas no difference in classification of negative words was observed. In the study of MDD and anxiety disorders, the focus has been predominantly on processing of mood congruent or disorder specific information. The shared abnormality as demonstrated in this study may be linked to the increased (negative) self-focus that is associated with both depression and anxiety (Borden, Lowenbraun, Wolff, & Jones, 1993; Spurr & Stopa, 2002; Watkins & Teasdale, 2004). Processing of self-referential material, for example, has been associated with increased memory success (Symons & Johnson, 1997). In a sad emotional state, negative words may be more relevant for the self than positive words, and therefore self-relevant information is more likely to affect the negative mood state. As a result, positive words may be considered less salient and as a consequence are processed in a less efficient manner. This less efficient processing of positive information may be reflected in the words classification behavior, prolonged response times and the reduced hippocampal activation during positive encoding. Consequently, positive information may be less potent in affecting the current mood state, thereby influencing the course of the disease in a negative way as well. However, the influence of a negative self-focus on processing of external positive information and the role of the hippocampus therein needs to be further investigated.

These results underline the importance of abnormalities in processing positive information in MDD and anxiety disorders and further emphasize that next to the traditional focus on mood-congruent processing abnormalities, mood-incongruent processing abnormalities should also be considered. Not only in the study of MDD, but also in the study of anxiety disorders. Recently, evidence was provided that patients with MDD have difficulties in engaging in positive mood states for prolonged periods of times, as was reflected in a failure to sustain activity in the nucleus accumbens (Heller et al., 2009), which is an important structure implicated in the reward system. Whether such a mechanism generalizes to patients with (comorbid) anxiety disorders, remains to be elucidated.

MDD ≠ ANXIETY DISORDERS

Besides the shared characteristics, distinct neuroanatomical and neurophysiological abnormalities were observed in MDD and anxiety. These unique characteristics could help explain the specific symptomatology of the disorders.

MDD patients, but not patients with anxiety disorders with or without MDD, showed lower volume of the right inferior frontal gyrus compared with controls. The inferior frontal gyrus region has been associated with processes of selective attention, cognitive conflict resolution, goal maintenance, and emotional inhibition (Forstmann et al., 2008; Lieberman et al., 2004; Matthews

et al., 2009; Ochsner et al., 2009; Phillips et al., 2003a). Interestingly, in our study, prefrontal abnormalities during executive processes were only observed in moderately and severely depressed patients and not in patients with remitted or mildly depressed states, nor in patients with current anxiety disorders. Although connectivity was not investigated in patients with anxiety disorders or comorbid depression-anxiety disorders, our results of decreased connectivity of the ventral striatum and the ventral medial PFC within the salience network during the execution of an emotional word classification task further suggests that top-down control of prefrontal regions over ventral emotion generating regions is compromised in MDD. Importantly, the specificity of this finding for MDD needs to be tested. In this study, we did not test for the directionality of cortical-paralimbic connectivity. However, dorsal prefrontal regions have been uniquely associated with top-down emotional regulation (Ochsner et al., 2009).

Moreover, moderately and severely depressed MDD patients showed increased responsiveness of the amygdala, striatum, and PFC during encoding of negative words. This may indicate an increased sensitivity for negative information in general that is specific to MDD in the 'active' depressed state, because it was not observed in remitted and mildly depressed MDD patients nor in patients with anxiety disorders. Also, MDD patients showed depressive state dependent ACC hyperactivation during encoding of positive words and state-independent increased activation of the left insula during encoding of negative words. Together these results indicate that the increased 'sensitivity' of limbic and prefrontal regions for negative information and increased attentional control during positive encoding is primarily associated with depressive pathology. Furthermore, these observations are in line with the suggestion that MDD and MDD with comorbid anxiety disorders should be considered different diagnostic categories.

In summary, MDD is uniquely characterized by inferior frontal volume reduction, state dependent dorsolateral PFC activation abnormalities as a function of planning load, increased sensitivity for encoding negative information, and by an increased demand for attentional control during encoding of positive words as reflected in increased ACC activation. The latter phenomenon may be reflective of a conflict resolution between the personal mood state and the emotional valence of the words presented during the task. Together these unique characteristics indicate more deficient involvement of dorsal frontal regions in MDD than in anxiety disorders, together with increased sensitivity for general negative information.

Structural abnormalities specific for anxiety disorders were observed as well. Patients with a current diagnosis of anxiety, but not those with a concurrent diagnosis of MDD, showed decreased volume of the superior temporal gyrus volume. This may mark a neuroanatomical vulnerability related to impaired evaluation of interoceptive information (Uchida et al., 2008) and altered threat processing (Phillips et al., 1998). These processes may be more specific for anxiety disorders than for MDD. During recognition of positive words, patients

with anxiety disorders showed increased activation in the left inferior frontal gyrus. No subcortical functional abnormalities were observed in patients with anxiety disorders in this study, which may be related to the more general tasks that were administered to investigate emotional processes: no specific (social) threat stimuli were included in the functional imaging paradigms. The use of social or situational threat paradigms may better highlight anxiety specific abnormalities, as the symptomatology of anxiety disorders is often more situational bound that in MDD. However, fearful faces were presented in the emotional faces paradigm, a task that was included in the NESDA neuroimaging study but not subject of this thesis. Analysis did not reveal functional abnormalities during viewing of fearful faces in patients with anxiety disorders, nor in the other patient groups (Demenescu et al., 2010). To conclude, our results suggest that outpatients with prevalent anxiety disorders are not functionally affected during executive task execution and processing of general negative information.

COMORBID DEPRESSION-ANXIETY VS. MDD AND ANXIETY

Contrary to our expectations, patients with comorbid depression-anxiety showed structural and functional abnormalities similar to patients with only MDD or anxiety disorders. This is surprising, considering the higher symptom severity in comorbid depression-anxiety on both measures of depression and anxiety than in 'singular' (i.e., without comorbidity) MDD and anxiety. Because comorbid depression-anxiety is associated with worse outcome, we expected to see this reflected in the neuroanatomical and functional patterns. It is possible that the currently used paradigms in combination with the current analysis approaches were not sensitive enough to detect differentiating characteristics in comorbid depression-anxiety. Nevertheless, patients with comorbid depression-anxiety were not characterized by comorbid depression-anxiety specific abnormalities, but by abnormalities in rostral ACC volume and right hippocampal reactivity during positive word encoding similar to patients with singular MDD and anxiety disorders. In the moderately and severely depressed state, patients with comorbid depression-anxiety were characterized by increased fronto-polar recruitment when recognizing previously encoded emotional words, independent of valence. As a function of visuospatial planning only trend-wise increased dorsal PFC activation was observed. Together these results might suggest that comorbid depression-anxiety patients are characterized by increased frontal recruitment during attentional demanding tasks only in the presence of emotional distraction or content, and only in the severely depressed and anxious state. However, at the moment, this suggestion is tentative and needs further investigation.

Volume reductions in the inferior frontal gyrus were observed in patients

with MDD only, and not in patients with comorbid depression-anxiety. This difference in result could be the result of a difference in aetiology in MDD and comorbid depression-anxiety: in most comorbid depression-anxiety patients, the anxiety disorder was the first to manifest. Possibly, anxiety related pathology is the primary pathology, and the occurrence of the comorbid depression later on only minimally affects the primary neuropathology. Interestingly, this explanation does not clarify why the superior temporal gyrus volume abnormality was only observed in anxiety without MDD and not in comorbid depression-anxiety. However, these findings indicate that anxiety and anxiety with comorbid MDD are not characterized by an identical neurobiological profile. These differences might explain the syndrome specific characteristics.

In sum, no evidence was provided for the suggestion that comorbid depression-anxiety is associated with more severe structural and functional abnormalities than patients with singular MDD or anxiety disorders on the measures included in this study. Although not subject of this thesis, fMRI was also applied during an emotional face viewing task data in the NESDA neuroimaging study. Analyses also failed to demonstrate differences between patients with comorbid depression-anxiety and patients with singular MDD and/or anxiety disorders in amygdalar response to negative emotional faces (Demenescu et al., 2010). Nevertheless, on measures presented in this thesis, comorbid depression-anxiety showed overlap with the functional and structural pattern observed in both anxiety disorders and MDD, but no overlap with MDD specific abnormalities was observed. Analysis of symptom course trajectories of the NESDA sample suggests that patients with anxiety disorders and comorbid depression-anxiety disorder are characterized by lower recovery rates and a higher proportion of patients with a chronic course than MDD patients without comorbid anxiety disorders (Penninx et al., 2010). The present neuroimaging results further suggest that patients with comorbid depression-anxiety disorders are not primarily characterized by MDD related pathology. Whether comorbid depression-anxiety is better characterized by anxiety related pathology on a neurobiological level should be studied further.

STATE VS. TRAIT

In this study, all patients fulfilled criteria for MDD, panic disorder, social anxiety disorder or generalized anxiety disorder in the last six months (plus scan interval). Remission as discussed in this thesis should therefore be considered 'recent remission' and caution should be taken when discussing our observations in light of proper state vs. trait related phenomenon. In this study, rostral ACC, inferior frontal gyrus, and superior temporal gyrus volume was observed independent of illness severity. Whether normalization of regional brain volume occurs in the absence of symptoms for a longer period

of time could not be tested in this cross-sectional study. Longitudinal analyses should confirm the hypothesis that reduced volume in these regions is a trait phenomenon of depression and anxiety disorders and therefore most likely constitutes a common vulnerability for a new anxiety- or depressive episode. The only longitudinal volumetric study performed in MDD to date indicates that remitted patients show less volume decline than non-remitted patients after a 3 year stable remission period (Frodl et al., 2004). Importantly, no volume 'normalization' was reported in remitted patients (Frodl et al., 2004).

Depression and anxiety severity related abnormalities were observed during tasks as measured with functional MRI. For example, in MDD prefrontal over-recruitment during planning, increased amygdalar, striatal and prefrontal cortex activation during the encoding of negative words, and increased dorsal ACC activation during the encoding of positive words, was found to be specific for the moderately and severely depressed state. Trait-like effects were observed during the encoding of positive and negative words in MDD, but not as a function of planning load. Hippocampal blunting during positive encoding and the insular hyperactivation in MDD during negative encoding were observed independent of current symptom severity. In anxiety disorders, hippocampal hypoactivation during encoding of positive words and inferior frontal hyperactivation during recognition of positive words were observed, although the latter was trend-wise associated with severity of anxiety. Relative decoupling of the ventral striatum and medial prefrontal cortex with a task executive network in MDD during classification of emotional words was observed independent of depression severity as well. Whether these trait-like effects persist after six month of full and stable remission should be studied using a longitudinal analysis approach.

Taken together, our results indicate that MDD, comorbid depression-anxiety, and anxiety disorders without MDD are characterized by trait like abnormalities that may constitute a vulnerability factor in patients who recovered from an anxious or depressive episode. However, the stability of effects need to be confirmed in longitudinal analyses, for example with the use of the longitudinal imaging data that are obtained in the context of the NESDA neuroimaging study. These data are currently available and analyzed. State related abnormalities, on the other hand, may reflect the episode specific symptoms, such as increased negative focus on the self and increased attentional problems in MDD.

THE ROLE OF RISK FACTORS IN NEUROANATOMY OF MDD AND ANXIETY

Early life experiences and personality characteristics were related to volume in regions associated with emotion perception and regulation, next to abnormalities related to current psychopathological status. Remarkably, the

experience of two or more incidents of psychological trauma or emotional neglect was associated with lower volume of the dorsal medial prefrontal cortex compared with participants who reported no incidence of any type of trauma in their lives. This volumetric abnormality was observed independent of current psychopathological state and is most likely associated with an early disruption of the human stress system in the developmental phase. These results indicate that vulnerability for developing mood- and anxiety disorders after exposure to early life trauma may be mediated by a neuroanatomical abnormality in regions associated with regulation of stress response. Next to regulation of stress responses, the dorsomedial PFC has been associated with the regulation of autonomic responses and regulation of arousal associated with emotional states and behavior (Phillips et al., 2003a; Urry et al., 2009). Abnormal morphometry of this region could therefore affect normal affective regulation, and could enhance sensitiveness for mood- and anxiety disorders.

In addition to these abuse related effects, we found that extraversion was associated with volume of the orbitofrontal cortex and orbitofrontal cortex/subgenual ACC. These regions have been associated with the gating of information in- and outflux from the amygdala (Price, 2003) and with control over amygdalar responsiveness (Milad & Rauch, 2007). The orbitofrontal cortex (including the subgenual ACC) could therefore be considered an visceral regulatory component, in line with the Mayberg model (1997). Furthermore, extraversion was positively associated with volume of the amygdala, a key area in salience detection and processing of emotional cues. Even though we only studied variation of the personality factors neuroticism and extraversion and only in healthy control participants, these results underline that personality factors are associated with morphometry of areas important for emotional perception and regulation. High levels of neuroticism have been associated with an increased risk for develop mood and anxiety disorders (Bienvenu et al., 2004), whereas high levels of extraversion have been found to act as a protective factor for developing mood and anxiety disorders (Clark et al., 1994). In this context, the present results suggest that this vulnerability may be mediated by personality dependent variations in orbitofrontal volume. In a functional connectivity study, also based on NESDA neuroimaging data, neuroticism has been shown to influence connectivity of the ACC and amygdala during negative face viewing (Cremers et al., 2010). Although the authors controlled for variations in extraversion, they did not test for the direct effect of extraversion on amygdala connectivity.

Alternatively, the present results could be interpreted as being reflective of variations in depression and anxiety related symptomatology in healthy subjects. Neuroticism has been shown to correlate positively with symptoms of depression and anxiety, even in the general population, whereas extraversion was found to be negatively correlated with symptoms of depression and anxiety in the general population (Jylha & Isometsa, 2006).Neuroticism and extraversion could therefore be regarded important personality dimensions

reflecting depression and anxiety related symptomatology. In this context, our results indicate that volume of subgenual regions vary as a function of this personality dimension (i.e., extraversion), even in healthy participants, and may reflect subsyndromal or normal variations in depressive and anxious symptomatology.

Interestingly, we also observed lower gray matter volume of the subgenual and orbitofrontal volume in patients with early age at onset of the first depressive episode in addition to the rostral ACC reduction that was observed in all patients (**Chapter 2**). Although we did not test for the correlation of extraversion in this region in patients with onset of MDD before the age of 18, these volumetric abnormalities suggest that the orbitofrontal region is an important region associated with vulnerability for developing depression and anxiety disorders. Given the numerous connections of this subgenual ACC/OFC regions with other important regulatory regions, this may also explain the heightened vulnerability for developing mood and anxiety in patients at high risk and the worse course of patients with early onset of depression.

In summary, risk factors associated with MDD and anxiety disorders were associated with volumetric variation in structures that have been linked to emotional perception and emotional regulation. Therefore, these factors are potent to influence the likelihood of developing mood and anxiety disorders. However, the exact causal mechanism from risk factor to affective disorder should be further investigated. Possibly, these factors set the homeostasis of the emotional regulation circuitry different, thereby making the brain circuitry associated with emotional and stress regulation more susceptible for dysregulation under stressful events, such as negative life events. The fact that childhood maltreated patients with MDD and/or anxiety disorders were characterized by the experience of more negative life events supports this suggestion (**Chapter 6**).

CAUSE OR EFFECT?

In this study, we investigated the relation between current psychopathology, emotional childhood abuse, and personality factors on one hand, and brain function and structure on the other hand using a cross-sectional design. Although early life factors such as early onset of depression and the experience of childhood emotional maltreatment appear to have a profound influence on brain morphometry, no conclusions with respect to cause and results can be drawn from the studies presented in this thesis. It is plausible though, that childhood emotional trauma would lead to abnormalities in the 'settings' of the human stress system, thereby affecting neurogenesis of regions that are rich in glucocorticoid receptors and are important for emotional control. However, this statement on causality can't be derived from the current study design. A current diagnosis of depression and/or anxiety disorder was associated with

volumetric and activation abnormalities as well. Whether these abnormalities are causal to the symptoms associated with MDD and anxiety disorders remains unclear. In our voxel based morphometry study, no relation between the number of episodes and volume of the rostral ACC or volume of the hippocampus was observed, suggesting that multiple episodes of depression do not aggravate the neuropathology. Again, however, the cross-sectional study design does not allow to infer on cause and result relationships and to study long-term effects of disease factors within patients. Longitudinal analyses should give insight into the causality of the relationship. To date, longitudinal imaging studies in MDD and anxiety disorders are virtually non-existent, with the exception of the volumetric studies of Frodl et al. (2004). Moreover, no longitudinal analyses in at risk populations have been performed that could answer the cause or effect questions.

COGNITIVE PERFORMANCE IN MDD AND ANXIETY

Contrary to our expectations, patients with MDD, comorbid depression-anxiety, and anxiety disorders showed no gross abnormalities in task performance during the visuospatial planning task and during the emotional word memory task. Only two cognitive tasks were administered, and therefore only little can be concluded about the status of neuropsychological functioning in MDD and anxiety disorders. The present results of relatively normal cognitive executive performance and absence of mood-congruent memory biases could be explained by the methods used to assess cognitive functions and by the moderate symptom severity of the outpatient sample included. Both options and implications of our findings will be discussed.

Behavioral results of the Tower of London task indicated only subtle slowing in the most depressed MDD patients during execution of the most difficult trials, in the context of normal planning accuracy. It could be argued that this computerized version was not sensitive enough to detect differences between groups, possibly owing to the two-answer option provided (as opposed to open-end questions/trials). However, in other psychiatric groups, this version of the Tower of London was sensitive enough to detect abnormalities in task performance (van den Heuvel et al., 2005a). Additionally, no effect of diagnosis on overall memory performance was observed in the present study. Nevertheless, performance differences were observed with the use of the same task in participants at high risk for developing depression or anxiety (Wolfensberger et al., 2008), suggesting the task is sensitive enough to detect performance differences. In this study, the retention interval was relatively short. Lengthening the retention interval or including a free-recall session could increase the sensitiveness of the test to detect subtle cognitive biases or deficits. Also, no effect of diagnosis on memory performance of mood-congruent or mood-incongruent words was observed. Possibly, words presented during the encoding phase were not salient enough to influence memory formation. Also,

presented words were not disorder-specific and therefore may not have been potent to affect memory formation. No depression or anxiety specific words were presented: instead words had a genera;;u negative, neutral, or positive connotation.

Because cognitive performance was also unaffected in the most severely anxious and depressed patients in the present sample, an alternative explanation is that cognitive functions are relatively unaffected in outpatients with MDD and/or anxiety disorders. Our results of normal cognitive performance in MDD and anxiety disorders, although sparingly assessed, are in line with a number of neuropsychological studies in outpatient samples that reported normal neuropsychological functioning in MDD and anxiety (Castaneda et al., 2008; Castaneda et al., 2010; Gladsjo et al., 1998; van Wingen et al., 2009). Our results of relatively unaffected planning and memory performance support the suggestion that cognitive capacities are not diminished in MDD and anxiety. Our results of slightly longer response times and increased dorsolateral PFC activation in the most difficult trials may be reflective of the narrowed attentional focus hypothesis instead (in: Hartlage et al., 1993): extra frontal resources were recruited to focus on external task demands that were not depression-relevant. Moreover, emotional words did not affect memory performance in a mood-congruent of mood-incongruent manner. Nevertheless, abnormal activation of limbic areas was observed during encoding of emotional information. Combined with the observation of slower encoding of positive words, our results suggest that positive information is to a lesser extent linked to (less) available positive material. Thereby, positive information could be less potent in affecting mood in a positive manner.

Discrepancies in results with studies that reported cognitive deficits in MDD and anxiety disorders (Airaksinen, Larsson, & Forsell, 2005; Basso et al., 2007; Paelecke-Habermann, Pohl, & Leplow, 2005; Porter et al., 2003; Purcell et al., 1997; Rose & Ebmeier, 2006; Tavares et al., 2007) may lie in differences in clinical characteristics. In this study, all patients were recruited in the outpatient setting, were never hospitalized for their psychiatric disorder, were relatively young (all aged under 57), did not use any antidepressant medication other than SSRIs at the time of the scanning session, were moderately depressed on average, and were free of a diagnosis of alcohol abuse. Our findings are therefore unlikely affected by negative side effects of, for example, sedative drugs on visuospatial capabilities and concentration (Golombok, Moodley, & Lader, 1988). Instead, our findings may be representative of the on average mildly depressed outpatient sample.

Interestingly, cognitive dysfunction could contribute to the functional impairment experienced by the patients and could therefore constitute a predictor or reason for hospitalization (Elliott, 1998; Jaeger et al., 2006; Withall et al., 2009). Neurocognitive findings obtained in the inpatient setting may therefore not necessarily generalize to the outpatient population. Elliott et al. (1996) demonstrated that inpatient performed worse on several

neuropsychological test in a direct comparisons of MDD in- and outpatients. Importantly, the in- and outpatients did not differ on any clinical variable, including illness severity. In addition, Purcell et al. (1997) reported that in a group of young MDD outpatients, those with a history of hospitalization performed worse on executive tasks. Together, these findings suggest that cognitive dysfunction is not merely a symptom of the disease, but could be a predictor of clinical care utilization.

IMPLICATIONS FOR NEUROANATOMICAL MODELS OF MDD AND ANXIETY

ANTERIOR CINGULATE CIRCUITRY

Our results emphasize the importance of the rostral ACC for both MDD and anxiety disorders. The rostral ACC has been implicated in the cognition-emotion integration hub, also including the medial PFC and orbitofrontal gyrus (Mayberg, 1997). This structural abnormality is likely to affect brain function, although the relation between structural and functional abnormalities in psychiatric disorders is barely studied. However, the importance of the rostral (or pregenual) ACC region for adaptive emotion regulation has been emphasized in the study of both MDD (Mayberg, 1997; Seminowicz et al., 2004) and anxiety disorders (Etkin, Egner, Peraza, Kandel, & Hirsch, 2006). Our results support the suggestion of a shared neuropathology in MDD and anxiety disorders, and indicate that this rostral or pregenual ACC subdivision should be considered for a general neuroanatomical model of psychopathology.

Functionally, the rostral ACC has been less frequently associated with depressed or anxious psychopathology than the subgenual ACC, a more ventral ACC locus. However, abnormal BOLD responses in the pregenual ACC have been observed and linked to altered glutaminergic metabolism in MDD (Walter et al., 2009). Deep brain stimulation of the subgenual ACC has been associated with immediate symptom improvement on symptoms such as restlessness, mood, interests, and motivation, even in treatment resistant MDD (Lozano et al., 2008). Also, this subgenual locus has been associated with treatment response in social anxiety disorder (Furmark et al., 2002). This may indicate that the subgenual ACC is a target area for successful treatment, but could also be considered an important starting point to understand the neuropathology of MDD and anxiety disorders.

A diffusion tensor imaging study that applied probabilistic tractography, a technique that calculates the direction of white matter tracts from any chosen seed, demonstrated that the subgenual and pregenual ACC are distinct regions in terms of structural connectivity (Johansen-Berg et al., 2008). Although both ACC subregions were found to project on the dorsal ACC, frontal poles, hypothalamus, and nucleus accumbens in healthy participants, the pregenual ACC was shown to have the strongest connections with the medial PFC and

dorsal ACC. The subgenual PFC on the other hand was most strongly connected to the amygdala, anterior hippocampus, nucleus accumbens, hypothalamus, and orbitofrontal cortex. Therefore, stimulating the subgenual ACC most likely influences the subcortical and (para)limbic emotion appraisal and generation regions, and could explain the immediate improvements in mood and motivation. In fact, deep-brain stimulation of the subgenual PFC decreased metabolism in the orbitofrontal cortex and medial PFC, also after six month follow up in treatment responders (Lozano et al., 2008).

Importantly, the pregenual ACC is anatomically connected to the dorsal ACC (Johansen-Berg et al., 2008). Functionally, the dorsal ACC has been found to influence amygdala activity negatively, both direct and via the posterior cingulate gyrus (Stein et al., 2007). In this thesis, we also described lower volume in the posterior cingulate gyrus in MDD and anxiety disorders, although below threshold. This finding suggests that connectivity of the dorsal ACC with the amygdala may be compromised in two ways: as a result of lower volume of the rostral and dorsal ACC, and as a result of lower posterior cingulate volume. Moreover, the pregenual ACC was found to inhibit amygdala activation during an emotional task in healthy controls, a process that was associated with emotional conflict resolution (Etkin et al., 2006). In treatment studies of MDD, the pregenual ACC was found to differentially influence metabolism in the subgenual ACC, orbitofrontal cortex, and dorsolateral PFC, depending on successful or unsuccessful treatment (Seminowicz et al., 2004). Finally, the pregenual ACC has been found to show abnormal connectivity with the anterior insula in MDD, dependent on illness severity (Horn et al., 2010). To sum up, the pregenual ACC appears an important region that is interconnected with a large number of other important regions implicated in neuroanatomical models of affect regulation. As it appears, the pregenual and subgenual ACC regions should both be considered important visceral regulation areas and should be considered at the center of an 'emotion regulation circuitry' affected in MDD and anxiety disorders.

In this context, our finding of reduced dorsal and rostral ACC gray matter (extending into the subgenual regions and adjacent white matter volume) in MDD indicates that communication with the dorsal ACC, subgenual ACC, orbitofrontal cortex, and medial PFC may be compromised in outpatients with MDD and/or anxiety disorders, leading to a range of symptoms. This suggestion is further supported by our findings of decreased connectivity of the medial PFC and ventral striatum with a salience network involving dorsal prefrontal regions, including the dorsal ACC, dorsomedial PFC, ventrolateral PFC, and dorsolateral PFC, although unrelated to volume of the rostral ACC. However, we did not investigate the connectivity within this task executive network in patients with anxiety disorders or comorbid depression-anxiety, and therefore the generalizability of this result to anxiety and comorbid depression-anxiety remains unclear. Previous studies have emphasized the importance of the medial PFC in both mood regulation through cognitive appraisal (Johnstone

et al., 2007; Ochsner et al., 2009), self-referential processing (Lemogne et al., 2009), and affective conflict processing (Ochsner et al., 2009). In summary, abnormal mood and anxiety regulation in MDD and anxiety disorders may be the result of compromised communication between rostral ACC - medial PFC, rostral ACC – dorsal ACC, dorsal ACC – amygdala, dorsal ACC – posterior cingulate gyrus – amygdala, amygdala – hippocampus, amygdala – subgenual ACC – orbitofrontal gyrus, originating from a common volumetric abnormality of the rostral ACC. Functional connectivity and structural connectivity studies should test whether deficient connectivity between these regions is linked to lower volume of the rostral ACC.

LATERAL FRONTAL AND TEMPORAL CIRCUITRY

In addition to the proposed common neuropathology, inferior frontal gyrus and the lateral temporal regions may moderate the emotional circuitry in a disorder specific manner. For example, Brodmann 45 in the inferior frontal gyrus, or ventrolateral PFC, projects on the dorsolateral PFC, medial PFC, orbitofrontal cortex (Price & Drevets, 2010). It has been suggested that the ventrolateral PFC could be regarded a multi-modal cortex which connects to circuits responsible for emotions as well (Price & Drevets, 2010). In the context of mood regulation in MDD, the ventrolateral PFC has been associated with increased activation when reappraising negative stimuli and abnormal engagement in a ventrolateral PFC - ventromedial PFC – amygdalar circuitry (Johnstone et al., 2007). This circuitry is associated with effort to down-regulate negative emotions. Therefore, abnormal volume of the right inferior frontal gyrus in MDD could contribute to this abnormal circuitry and affect the capability to regulate negative emotions. Additionally, increased left dorsolateral PFC activation was observed as a function of increasing planning load in moderately and severely depressed MDD patients, suggesting that even in the context of emotional non-demanding task demands, extra frontal resources are called upon to focus on external task demands. This finding may indicate that the dorsolateral PFC in the active depressed state is less potent in controlling dorsolateral PFC dependent emotion regulation. The superior temporal gyrus on the other hand, projects to the medial prefrontal circuitry as well (Price & Drevets, 2010), and thereby could affect medial PFC linked emotional regulation as well.

MEDIAL TEMPORAL AND LIMBIC CIRCUITRY

Although frequently associated with abnormal processing of emotional stimuli in MDD and anxiety disorders, only modest abnormalities in amygdalar function were observed. No volumetric differences were found in the amygdala. Abnormal activation was demonstrated in moderately to severely depressed MDD patients only during the encoding of negative words. Analysis of the emotional faces data acquired in the context of the NESDA neuroimaging study, although not subject of this thesis, failed to provide evidence for the suggestion that abnormal amygdalar and subcortical signaling in response to

sad and fearful faces is at the base of the disorders (Demenescu et al., 2010). However, the fact that no gross between group effect was observed in the amygdala in anxiety and comorbid depression-anxiety, does not rule out the amygdala as an important structure in the pathophysiology of depression and anxiety. For example, in an emotional conflict resolution task, the rostral ACC was found to inhibit amygdalar response in healthy controls (Etkin et al., 2006), whereas this inhibitory effect was absent in patients with generalized anxiety disorder (Etkin et al., 2010).

In the NESDA study, connectivity during 'resting state' was studied in first episode MDD patients. Veer et al. (2010) reported decreased connectivity of the amygdala and the frontal poles with an affective network including the anterior insulae, dorsal ACC, ventromedial PFC, temporal poles, and amygdalae. Also, using NESDA neuroimaging data, Cremers et al. (2010), demonstrated that connectivity of the right amygdala and the medial PFC during negative face viewing is positively correlated with neuroticism scores, whereas connectivity of the left amygdala and the dorsal ACC was found to correlate negatively with neuroticism in healthy controls. As mood and anxiety disorders are associated with high levels of neuroticism, it is likely that amygdalar-dorsal PFC connectivity maybe be altered in patients with MDD and/or anxiety disorders compared with controls. Finally, the orbitofrontal and subgenual anterior cingulate cortex may also affect amygdalar – medial PFC/ACC connectivity, as neuroticism was also found to be positively correlated with orbitofrontal and subgenual ACC volume (**Chapter 7**). Given the direct links between the orbitofrontal cortex and subgenual ACC on one hand and the amygdala on the other hand, neuroticism might further modulate amygdala – ACC connectivity via the orbitofrontal cortex. In conclusion, it is possible that amygdalar abnormalities become more evident and meaningful when studied in relation to activation of other regions.

In this, the hippocampus was only found to be functionally affected in MDD and anxiety disorders: hippocampal blunting was observed during positive word encoding. This result suggest that positive processing is altered in MDD, and most likely reflects diminished hedonic capacity. This suggestion was further supported by our finding of decreased connectivity of ventral striatal, including the nucleus accumbens and medial PFC areas within a salience network, related to classifying positive words. However, we could not test the effect of hedonic capacity in this study, as no measure of state anhedonia was administered on the day of scanning. Nevertheless, neural circuitry in MDD and anxiety could differ along a dimension of affective state, such as sad mood or lack of positive affect. This suggestion is supported by recent work, where an inability to maintain nucleus accumbens activation over time during positive affect regulation was related to individual differences in anhedonia in MDD patients (Heller et al., 2009). The nucleus accumbens is an important region in the human reward circuitry and these results suggest that the reward circuitry fails during positive affect regulation in MDD. Whether inter-individual variations in other depression or anxiety specific dimensions, such as sad mood or anxious

arousal, are related to a specific neural circuitry as well should be studied more extensively.

In summary, implications for models of emotional regulation in MDD and anxiety based on results presented in this thesis are 1) the rostral ACC should be implicated in a general model of affective disorders, as it is potent to influence a wide variety of regions implicated in normal emotional regulation; 2) the inferior frontal gyrus, dorsolateral PFC, and superior temporal gyrus could affect the emotional circuitry in a disorder specific way; 3) variations in regional brain volume related to risk factors could contribute to the understanding of the complex neuropathology of depression and anxiety disorders; 4) current mood state could influence the dynamics of the mood circuitry, as valence specific functional abnormalities were observed.

SAMPLE CHARACTERISTICS

The NESDA neuroimaging study is the first structural and functional MRI study implemented in a large observational and longitudinal framework. The NESDA study is unique in that it includes both measurements of phenomenological as well as biological factors and aims to integrate psychosocial and biological models in order to examine predictors of the long-term course and consequences of MDD and anxiety disorders. The major advantage of large-scale observational cohort studies is the possibility to include a large and well-characterized sample of participants with a large variety of symptoms, comorbidity, personal history, and other 'real life' characteristics. This allows epidemiologists to identify patterns of psychopathology related to biological factors, aetiology, treatment response, and outcome. Nevertheless, some issues should be considered.

First, since the epidemiological NESDA cohort was recruited through general practitioners and outpatient clinics, we may not have been fully able to capture the most severe end of the depressive and anxious spectrum. Also, patients that first participated in an intensive interview session and subsequently were willing to participate in a two-hour MRI- and interview session, may not be most representative of the MDD sub-sample with large motivational problems.

Furthermore, because in NESDA the focus is on MDD, dysthymia, social anxiety disorder, panic disorder, agoraphobia, and generalized anxiety disorder, other modules of the Composite International Diagnostic Interview (CIDI) were not included in the interview such as those of posttraumatic stress disorder, obsessive-compulsive disorder, eating disorders, bipolar disorder, and attention hyperactivity disorder. Although, participants with a formal diagnosis of a psychiatric disorder not subject of NESDA were not included in the NESDA sample, it is possible that a percentage of participants included in NESDA neuroimaging sample formally fulfilled diagnosis for a psychiatric disorder that is associated with specific brain abnormalities.

Related to this issue, the healthy controls included in this study were also

obtained from a 'real life' sample. All were life time free of a diagnosis or subthreshold diagnosis of MDD or anxiety disorders, but a portion of the healthy controls were recruited from an at-risk population, namely from the Adolescents at Risk for Anxiety and Depression (ARIADNE) cohort study. The ARIADNE study focuses on the offsprings of patients with mood- and anxiety disorders. Also, for healthy controls recruited through the general practitioners, having a first-degree relative with a psychiatric disorder was not an exclusion criterion. Several neuroimaging studies focusing on cognitive and emotional processes in healthy subject, but also in psychiatric samples, included 'super' healthy control participants without a first degree relative with mood- and anxiety disorders or history of childhood trauma. The inclusion of including 'real life' healthy controls in NESDA had the major advantage of allowing us to study the effects of risk factors for developing mood- and anxiety disorders across patients and healthy controls, but may constitutes a source of discrepancy between our study and some published neuroimaging studies. A second advantage of this recruitment strategy is that our healthy controls were also recruited from NESDA, underwent the same screening as patients, and were scan-naïve. Therefore, in this study, familiarity with researchers and/or experimental procedures could be ruled out as a potential confound. In contrast, earlier studies included a control group consisting of, for example, staff members (Elliott et al., 1997; Fitzgerald et al., 2007; Okada et al., 2003). In these studies, it remains therefore unclear whether functional brain abnormalities are related to the psychopathology under study, or are related to the differences in state anxiety due to the novelty of the situation.

Finally, in this thesis, panic disorder, social anxiety disorder, and generalized anxiety disorder were studied in concert, as if these disorders are not characterized by syndrome specific abnormalities. However, panic disorder, social anxiety disorder, and generalized anxiety disorder have been associated with unique activation patterns in imaging studies and a unique neurobiology (Blair et al., 2008; Mathew, Coplan, & Gorman, 2001). Nonetheless, we did not aim to study anxiety disorder specific features, but concentrated on the shared neurophysiological features of prevalent anxiety disorders that frequently co-occur with MDD.

MRI

In the present thesis, magnetic resonance imaging was administered for measures of brain volumetry and brain activation related to executive and emotional processes. MRI could be used for other measurements as well, including. but not limited to, white matter tract integrity measurements and measurements of metabolites in brain tissue (e.g., glutamate). Other imaging techniques available have their specific advantages and disadvantages with respect to spatial and temporal resolution. In this section, considerations with respect to the currently administered imaging techniques will be discussed.

Although a wealth of information on brain tissue properties and oxygenation

differences is available in these scans, the techniques have their limitations. The MRI techniques used in this study are able to measure brain morphometry and activation at a spatial resolution of one or several millimeters and are capable to estimate volume and activation at this spatial resolution. The temporal resolution of fMRI in our study was 2.3 seconds, meaning brain tissue was imaged once in 2.3 seconds. Together with the spatial resolution of our fMRI sequence of 3 mm, observations in one 3D volume unit of 3 x 3 x 3 millimeter (1 voxel), this means that no microscopic observations can be made and that single cell firing or the net result of firing of groups of neurons at a resolution of milliseconds could not be recorded. In the case of BOLD-fMRI, we measured the net result in heamodynamic changes of a large number of neurons, related to neural firing (Logothetis,2002). Subsequently, we compared activation in one region during a certain condition to activation in that region to another condition, and therefore calculated a relative activation measure. In **Chapter 7**, we calculated the correlation of low frequency BOLD fluctuations as a measure of intrinsic connectivity. However, BOLD imaging can not distinguish between inhibitory and excitatory neural processes, and comparisons of absolute activity between two or more brain regions can not be made (Logothetis, 2002). Also, with the use of the currently used imaging techniques, no information on neurotransmitter concentration or activity was obtained. Disregulated neurotransmission is thought to play a major role in depression and anxiety disorders and likely affects regional neuronal activity or connectivity. Studying binding of pharmacological agents and neurotransmitter metabolism across the brain would be highly interesting and would provide important additional information on the neuropathology of MDD and anxiety disorders. However, such measurements could not be obtained with the techniques used in this study. In addition, the temporal resolution of functional MRI is poorer than for electroencephalography, and therefore does not allow to record very rapid neuronal firing. Nevertheless, MRI is one of the preferable techniques to study the brain, as it has the advantage of allowing studying both activation and structure in the same session. Moreover, fMRI allows studying activation deep in the brain, such as limbic activity, in a non-invasive way without the need to ingest a radioactive tracer.

CONSIDERATIONS FOR FUTURE OBSERVATIONAL MRI STUDIES

Structural MRI serves as an excellent tool to study the brain's morphometry in an observational set-up. Structural imaging techniques are minimally affected by day to day variations in symptomatology, medication use, time of scanning and site of scanning when similar MRI machines are used. Using functional MRI in an observational study is more problematic due to the large number of factors that is potent to influence the BOLD pattern. BOLD patterns are more likely to be influenced by factors such as time of day, quality of sleep the night before the scanning session, and subjective tension level during the scanning

session. Therefore, cross-sectional analysis could be insufficiently able to detect variability in BOLD patterns related to a current diagnosis owing to other structural noise factors.

For future observational functional MRI studies, a design that allows for an intra-subject challenge of subcortical/(para)limbic-cortical activity is recommended. For example, performing the Tower of London planning task before and after a positive or depressed mood induction session, or a pharmacological challenge, allows to study within-subject variability in response to a fixed experimental factor. Such a design would allow to study the flexibility or adaptability of the mood regulation circuitry within patients, a design that would be less sensitive to between subject factors such as quality of sleep, time of day etc. Monitoring within scanner variability over time and between-scanner variability is also highly recommended in order to get an estimate of the variability in the data due to scanner variability.

Finally, using the data obtained from the same sample for several publications is defensible considering the costs and effort taken by both researchers and participants. However, before setting up a large study, the maximal number of publications resulting from (sub) data sets should be determined, in order to set an appropriate alpha for considering significance for each report in order to sufficiently and appropriately control for multiple comparisons.

IMPLICATIONS

Our results suggest that MDD and anxiety disorders do not constitute distinct entities when considering their neuroanatomical and functional MRI profile, but instead share characteristics that may explain the maladaptive emotion regulation. In the case of comorbid depression-anxiety, researchers, and possibly clinicians as well, should keep in mind that the comorbid depression-anxiety is not simple MDD plus anxiety when considering structural and functional abnormalities related to emotional regulation. Instead, comorbid depression-anxiety might be better characterized by anxiety neuropathology as the primary neuropathology. For example, comorbid depression-anxiety disorder does not resemble MDD with respect to executive abnormalities and encoding of negative information. Therefore, in the study of regional volume or activation, this comorbidity of anxiety disorders may constitutes an important predictor instead of a confounder.

MDD and anxiety disorders appear to share functional and structural abnormalities, while at the same time, the disorders appear to be characterized by syndrome specific abnormalities. This pattern of both shared and unique characteristics seems to parallel the symptomatology of MDD and anxiety: large overlap in symptoms exists, but disorder specific symptoms are present as well. A more dimensional approach in characterizing both phenomenology and neurobiology of depression and anxiety disorders could aid to identify the shared neuropathology related to shared symptom dimensions. Such

an approach might also better capture the variability in neural abnormalities related to illness severity.

We demonstrated that childhood emotional maltreatment and variations in personality factors are also related to variations in volume of regions associated with emotional regulation. Although the anatomical correlates of emotional maltreatment was observed independent of psychopathological status, this aetiological factor could influence the neural circuitry normally responsible for adaptive emotion regulation. Variation in personality may also affect the neurocircuitry: orbitofrontal and subgenual ACC volume variations may affect normal emotional regulation. It is therefore recommended that future imaging studies do not consider early advert life events including childhood emotional maltreatment and variations in personality structure as confounds, but instead considered these factors as potentially important predictors of neurobiological variation in MDD and anxiety disorders.

Finally, symptom severity does not influence every aspect of brain structure and function as measured in the NESDA neuroimaging study. We observed shared structural and functional abnormalities in both MDD and anxiety disorders, even in the remitted state. This may further underline the importance of regular check-ups and 'maintenance' in patients with a (recent) history of depression and/or anxiety disorders. Training in focusing on positive events, for example in the context of mindfulness-based therapy may protect patients from relapsing into a new episode of depression and anxiety. However, the effects of such training on brain circuitry in remitted patients need to be further investigated.

SUGGESTIONS AND CHALLENGES FOR FUTURE STUDIES

SUGGESTIONS FOR FUTURE IMAGING STUDIES

In future neuroimaging studies that aim to get insight in the shared neuropathology of MDD and anxiety, connectivity of the prefrontal cortex and ventral regions should be studied in patients with anxiety disorders and directly compared to connectivity patterns in patients with MDD. In that way, the specificity of our finding of a decreased coupling of ventral striatal and medial PFC regions with a task executive network during execution of an emotional task could be tested. Related to this suggestion, studying subcortical-cortical connectivity during manipulation of mood or anxiety status, for example with the use of autobiographical scripts or pharmacological challenges, would provide important information. In this way the connectivity and the flexibility of the neural circuitry in different mood states could be tested. Such manipulations would tell us about the dynamics of the emotion generation and regulation system for several emotional states. Also, the brain circuitry involved in emotional regulation could be experimentally manipulated, for example with the use of neurostimulation techniques, thereby giving further insight in the dynamics on the neural circuitry and emotions experienced by the patient.

Furthermore, providing a challenge when studying cognitive processes in cross-sectional studies is important. Such a challenge could be a mood or stress induction session before a specific task. Also, challenges could be integrated in the task. For example, a mixed design including alternating blocks of executive trials without emotional distraction and executive trials with emotional distraction could be administered, thereby testing the limits of executive control in MDD and anxiety disorders. Such a design could be more sensitive for detecting emotional relevant disturbances in attentional control, as suggested by Wang et al (2008). Furthermore, inclusion of a mood and anxiety regulation task would be essential in the study of depression and anxiety.

Next, characterizing patients along dimensions of depression and anxiety related symptom dimensions, as proposed by dimensional models of psychopathology, could perhaps better capture the structural and functional correlates of current mood and anxiety status than a categorical characterization. In this study, we observed that inter-subject variability in factors such as personality dimensions was associated with regional brain volume in healthy controls. It is possible that using the between subject variation within patients in an more optimal way, by for example describing their symptomatology in a multi-dimensional space could help us further unravel the complex story of brain involvement in mood and anxiety disorders.

Finally, multivariate and multi-model approaches could help us find differentiating patterns of abnormal volume or activity in MDD and anxiety disorders. So far, univariate approaches have been predominantly used in the study of MDD and anxiety disorders, where a univariate test is performed per smoothed voxel. These methods may be less sensitive in detecting differentiating patterns in the highly integrated network of limbic-cortical regions. Multivariate analyses approaches are sensitive to spatially distributed information, whereas using univariate approaches test for between group differences per voxel. Using multivariate approaches could lead to the detection of highly differentiating features that could also be used in disease state prediction. Such multivariate approaches would both increase the sensitivity ánd specificity of functional and structural imaging findings. In addition, integrating imaging data acquired using different modalities, could help understanding the complex interactions of different regions. Combining information of regional volume, regional brain activation, metabolism, and functional and structural connectivity would be essential to understand the complex dynamics of adaptive and maladaptive mood regulation.

CHALLENGES: TREATMENT RESPONSE AND COURSE PREDICTION

A major challenge for future neuroimaging studies in MDD and anxiety disorders is to identify a set of features that could predict vulnerability for

developing MDD and anxiety disorders, illness course and treatment response. Currently, treatment response is unsatisfactory: up to 60% does not reach a good response (Ressler & Mayberg, 2007) and only a small percentage responds to mono-therapy with an SSRI. Being able to predict who will respond to mono-therapy with an SSRI or cognitive behavior therapy, or a combination of the two, would be very useful in clinical practice. Longitudinal studies will be very important for identifying such predictive features. Long-term outcome and treatment response could then be predicted from functional and structural MRI patterns, in combination with detailed information on symptomatology, occurrence of life events, medical and psychiatric comorbidity, family status of depression and anxiety, personality, psychological measures including dysfunctional belief systems, and occurrence of childhood maltreatment. So far, studies have indicated cognitive, structural, and functional features that were associated with treatment responses (Canli et al., 2005; Chen et al., 2007; Langenecker et al., 2007; Li et al., 2010). Symptom improvement in MDD have been associated with amygdalar response (Canli et al.,2005), ACC and insular volume (Chen et al., 2007), dorsolateral PFC volume (Li et al., 2010), and ACC activity (Chen et al., 2007). Including genetic variations could be very useful as well. Not only have genetic variations in, for example, the serotonin transporter gene been associated with the risk of developing mood- and anxiety disorders in the context of adverse life events (Caspi et al., 2003). Furthermore, the serotonin transporter gene has been associated with variations in amygdala response (Bertolino et al., 2005; Hariri et al., 2005; Lau et al., 2009), amygdala – ACC connectivity (Pezawas et al., 2005), and brain volume in (para)limbic regions (Frodl et al., 2008a) in healthy controls. Moreover, genetic variations have been related to treatment response, associated with responsiveness of the amygdala and ACC during emotional processing (Baune et al., 2010). Such multi-factorial models, including amongst others clinical, genetic, and neuroimaging information could then be used to provide an optimal treatment strategy for patients. As NESDA obtained information on all these measures, a first step in setting up such a model will be taken in the nearby future.