Using novel imaging approaches in affective disorders : beyond current models

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Chapter 7
Discussion

This thesis aimed to address gaps in the current knowledge of the underlying neurobiology of adults and adolescents with depression and anxiety, using innovative neuroimaging modalities such as resting-state fMRI and voxel-based morphometry to investigate brain structure and functional connectivity. Resting-state fMRI was used to study the functional connectivity patterns unique to SAD without comorbidity, and also, for the first time, to PD without comorbidity. These adult patients were directly compared to pair-wise matched healthy control subjects. Additionally, the shared and unique characteristics of MDD and anxiety disorders were examined in a comparison between adult MDD patients without comorbidity, anxiety patients without comorbidity, and patients presenting with comorbid MDD and anxiety disorder(s), compared to healthy controls. Anxiety disorders included in this study were PD, SAD, and GAD, and any combination of the three.
To address gaps in the knowledge on developmental features of affective disorders, resting-state fMRI and structural MRI were employed in a study on clinically depressed adolescents.

The studies in this thesis were based on samples derived from two longitudinal studies: the Netherlands Study of Depression and Anxiety (NESDA) and the Emotional Pathways' Imaging Study in Clinical Adolescents (EPISCA).
In this chapter, the results of the studies in Chapters 2 – 6 will be summarised, and their implications will be discussed.

Summary of results

The unique resting-state characteristics of PD were examined in Chapter 2. We demonstrated altered resting-state functional connectivity (RSFC) in the limbic and salience networks in a sample of PD patients without any other psychiatric comorbidity. For the limbic network, patients showed increased RSFC between the right amygdala and the bilateral precuneus and lateral occipital cortex. This effect may be linked to typical panic symptoms such as derealisation and depersonalisation. However, as associations between the effect and clinical severity were lacking, these speculations could not be confirmed. For the salience network, PD patients had decreased RSFC between the left dACC and the bilateral frontal pole and superior/medial frontal gyrus compared to healthy controls. In contrast, PD patients showed an increased RSFC with the bilateral

precentral and postcentral gyrus, right supplementary motor cortex, and right ACC. Furthermore, an increased RSFC between the right dACC and the right superior parietal lobule, lateral occipital cortex, angular gyrus, and central opercular cortex in PD patients was found. These findings could be related to panic symptoms such as a heightened awareness of bodily sensations, or disturbances in self-awareness that could be reflected in symptoms such as the feeling that one is going crazy or loses control (Pannekoek *et al*., 2013b).

In Chapter 3, RSFC in medication-naïve SAD patients without psychiatric comorbidity was discussed. For the limbic network, SAD patients showed increased negative right amygdala RSFC with the left middle temporal gyrus, supramarginal gyrus, and lateral occipital cortex compared to healthy controls. These areas have been linked to facial perception, and an aberrant RSFC could be interpreted along the lines of an enhanced sensitivity for facial expressions in SAD patients. For the salience network, increased positive connectivity was reported between the bilateral dACC seeds and the left precuneus and lateral occipital cortex in SAD patients. This could underlie a disturbed self-awareness and biased information gathering in SAD patients (Pannekoek *et al*., 2013c).

Chapter 4 discussed the shared and unique RSFC characteristics of substantial groups of MDD patients without comorbidity, anxiety patients without comorbidity, and comorbid depressed and anxious patients, compared to healthy control subjects. Using the data-driven independent component analysis (in combination with dual regression) approach, differences were found between the comorbid group and healthy controls in the limbic network, where comorbid depressed and anxious patients showed increased connectivity in a cluster containing the bilateral precuneus, intracalcarine cortex, lingual gyrus, and posterior cingulate, as well as a cluster including the right precentral gyrus, inferior frontal gyrus, and middle frontal gyrus. It was suggested that the aberrant RSFC pattern found in the current study is specific to comorbid depression and anxiety, only becoming evident when patients present with both disorders and not in patients with a single diagnosis of either (Pannekoek *et al., submitted for publication*).

Our fourth RS study was conducted in a sample of treatment-naïve adolescents with a clinical diagnosis of depression and pair-wise matched healthy controls. Chapter 5 describes the RSFC differences we found

between these groups. For the limbic network, depressed adolescents showed increased RSFC between the left amygdala and the right middle frontal gyrus, inferior frontal gyrus, precentral gyrus, and postcentral gyrus. These findings could be related to a disturbed cognitive control over emotion processing in adolescent depression. There was also an attenuated RSFC in the clinical group between the right amygdala and the left frontal pole, right ACC, paracingulate gyrus, and superior frontal gyrus, as well as with the left angular gyrus, lateral occipital cortex, and supramarginal gyrus. Altered connectivity between the amygdala and the ACC is typical for depression, and could be associated with abnormalities in emotion regulation. For the salience network, decreased RSFC was found in the clinical group between the dACC and the right middle frontal gyrus, frontal pole, and inferior frontal gyrus. Altered connectivity between the dACC and prefrontal areas may be associated with a bias towards negative emotional stimuli and disrupted assessment of affective stimuli (Pannekoek *et al*., 2014a).

In addition to RSFC in the previous chapter, grey matter volume in clinically depressed adolescents was studied in Chapter 6. A region-of-interest VBM revealed a smaller ACC volume by 14.4% in the patient group compared to pair-wise matched healthy controls. This is consistent with literature on adult depression. The ACC has been linked to higher cognitive functions, and problems with inhibiting the processing of negative material are common symptoms of depression. Abnormalities in grey matter volume of the ACC could be related to such problems. The emergence of psychopathologies in adolescence has been related to anomalies in brain maturation in combination with psychosocial, biological environmental factors. Our results may be interpreted as a result of abnormal maturational processes (Pannekoek *et al*., 2014b).

In sum, these findings point to the involvement of a wide array of brain regions in depression as well as in anxiety disorders that is not limited to a specific age range. The next sections will discuss our findings in light of neurobiological models and findings of contemporary studies of depression and anxiety.

Panic disorder

The most influential and extensive neuroanatomical model on PD was initiated by Gorman (1989; 2000). In his revised version of the original

neuroanatomical hypothesis, it was proposed that panic originates from an abnormally sensitive fear network including the insula, PFC, thalamus, amygdala, and projections from the amygdala to the brainstem and hypothalamus (Gorman *et al.*, 2000). Gorman's model was presented as a hypothesis, inviting further studies to confirm its assumptions.

In our RS study of PD patients without comorbidity, we focused on three RS networks that had been implicated in anxiety disorders before, but not in PD. With Gorman's neuroanatomical hypothesis in mind, the limbic network, probed with seeds in the bilateral amygdala, was particularly of interest. For this network, an increased negative RSFC was found with the bilateral precuneus and lateral occipital cortex. Whereas the amygdala plays a central role in Gorman's theory, the precuneus and lateral occipital cortex are not considered. With a strong focus on subcortical and limbic structures and the mPFC, the hypothesis does not include any posterior regions. The interpretation of our results that we offered was that the reported RSFC abnormalities could be associated with typical panic symptoms such as disturbances of self-processing and consciousness occurring during panic attacks. These functions are linked to the precuneus, which plays a role in self-reflection and self-processing activities like mental imagery and episodic/autobiographical memory retrieval. A heightened RSFC between the precuneus and the amygdala/limbic network, which plays a key role in emotional and fear processes, could be related to symptoms such as losing control, depsonalisation, and derealisation.

Similar to the precuneus and lateral occipital cortex, the regions found to have altered RSFC with the salience network are also new to models on PD. The left and right dACC showed altered RSFC with frontal and occipito-parietal areas that are involved in the processing of somatosensory information, attentional control, and self-awareness. With the salience network serving as an assessor of relevance of internal and external stimuli, an exaggerated significance attributed to such stimuli is much in line with the symptomatology of PD. Although Gorman notes in his revised model that misinterpretation of (benign) bodily signals is a hallmark of PD, and a potential deficit in the processing of sensory information is recognised as a contributor to the onset of panic attacks, brain areas involved in the appraisal of somatosensory input (e.g. precentral and postcentral gyrus) are not incorporated in the model.

The involvement of a more extensive neurocircuitry than what was described in the neuroanatomical model is also emphasised in other contemporary brain imaging studies. The past years have witnessed a substantial increase in the use of neuroimaging studies to further investigate the role of brain regions and circuitry that may be involved in the pathophysiology of PD (Pannekoek *et al.*, 2013a). De Carvalho and colleagues (2010) reviewed fMRI studies in PD, and included a discussion of treatment-related research (de Carvalho *et al.*, 2010). Functional studies show that brain structures such as the PFC, ACC, hippocampus, amygdala, and brainstem regions may play a major role in panic circuitry. The authors further stated that in particular the amygdala, PFC and hippocampus seem to be important for cognitive behavioural therapy (CBT) in PD, which serves to change dysfunctional thoughts regarding bodily sensations. On a neural level, reduced PFC activity could reflect an impaired top-down control of the fear response. Abnormal hippocampal activity may be related to an exaggerated appreciation of potentially threatening stimuli (de Carvalho *et al.*, 2010). PET studies point to the metabolic changes brought about by CBT in cortical areas such as the inferior frontal gyrus (IFG) (Prasko *et al.*, 2004) and the mPFC and hippocampus (Sakai *et al.*, 2006). Consistent with these findings, a recent fMRI study showed that CBT normalised a pre-treatment increase in IFG activation in PD patients compared to healthy controls, demonstrating an effect of CBT on neural mechanisms in PD (Kircher *et al.*, 2013). These studies provide further evidence for the involvement of cortical brain areas in PD that are not part of the neuroanatomical hypothesis.

Dresler and colleagues (2013) conducted a comprehensive literature review of PD studies, and discussed the findings in light of Gorman's model (Dresler *et al.*, 2013). Based on the existing literature and the ongoing gaining of insights by studies applying modern neuroimaging techniques, some amendments to the neuroanatomical model were suggested. The emphasis on the amygdala might be tempered, since findings on its involvement in PD are not univocal. Simultaneously, the role of several cortical areas such as the insula and ACC seems to be underestimated in the hypothesis. It is of note that the specific involvement of these hypothesised brain areas remains to be confirmed, as there is no conclusive evidence on the increase or decrease of regional volume and function (Dresler *et al.*, 2013).

In summary, our results are in line with other contemporary imaging studies on PD with respect to the neuroanatomical hypothesis from Gorman, suggesting the involvement of additional cortical areas in the pathogenesis of PD. It is evident that much more research is needed before a model can be proposed that is based on conclusive findings. Imaging genetics studies, experimental and longitudinal designs, and neurochemical approaches in future research could prove highly relevant for a better understanding of the neurobiology underlying PD.

Social anxiety disorder

Our resting-state study in SAD appeared to be a timely contribution to the research on the neurobiology of this disorder. Simultaneously with our publication, several other resting-state studies in SAD also came out. This increase in publications seems to parallel the accumulating interest in the neurobiology of SAD, one of the most prevalent psychiatric disorders.

We found altered RSFC between the right amygdala and the left middle temporal gyrus, supramarginal gyrus, and lateral occipital cortex in SAD patients. We also found increased RSFC between the bilateral dACC and the left precuneus and lateral occipital cortex. Our results are largely consistent with resting-state reports that appeared simultaneously with our study, of altered amygdala and ACC functional connectivity in SAD patients. One group found decreased RSFC in the primary somatosensory, motor, and visual networks in SAD patients. This group also reported increased RSFC in a medial prefrontal cortex network, and bidirectional abnormalities in a range of other networks (Liao *et al.*, 2010a). The same group showed an association between clinical severity and decrease of functional connectivity in the frontal and occipital lobe (Ding *et al.*, 2011). A third study by this group showed altered effective connectivity in SAD patients between the amygdala and the inferior temporal gyrus and visual cortices (Liao *et al.*, 2010b). Finally, the authors used regional homogeneity analysis to investigate RSFC in SAD patients, and reported decreased coherence in the angular gyrus and mPFC within the DMN in individuals with SAD (Qiu *et al.*, 2011).

Contemporary studies recognise the role of multiple brain regions in emotion, which is reflected in the use of neuroimaging modalities that highlight the involvement of neural networks rather than singular brain regions in SAD. Where resting-state fMRI studies address functional

connectivity of the brain, diffusion tensor imaging (DTI) is used to examine the structural integrity of white matter and to map white matter tracts. The uncinate fasciculus and superior longitudinal fasciculus have particularly shown involvement in the four DTI studies that exist in SAD. The uncinate fasciculus is a white matter tract that links the amygdala with the orbitofrontal cortex, and the superior longitudinal fasciculus connects occipital, parietal and frontal regions (Ayling *et al*., 2012, Fouche *et al*., 2013). Decreased fractional anisotropy was reported in the right uncinate fasciculus in SAD patients (Phan *et al*., 2009). Another study found decreased fractional anisotropy and volume in the left uncinate fasciculus and the left superior longitudinal fasciculus, and this decrease in fractional anisotropy was associated with higher trait anxiety in SAD patients (Baur *et al*., 2011). The same authors proceeded to find significantly reduced volume and fractional anisotropy at a trend level of the left uncinate fasciculus in SAD patients. Additionally, a decreased global mean fractional anisotropy was found in SAD patients (Baur *et al*., 2013). An increase in fractional anisotropy and volume of the genu of the corpus callosum was also reported (Liao *et al*., 2011). These studies suggest that distributed networks are involved in the mediation of anxiety in SAD (Fouche *et al*., 2013), and emphasise the importance of the connectivity between the amygdala and frontal cortical regions.

It is of note that despite this increased interest in the neurobiology of SAD, the entire imaging literature on SAD remains relatively small (Fouche *et al*., 2013). There is still no neuroanatomical model on SAD, like there is on PD. However, Rapee & Heimberg (1997) proposed their cognitive-behavioural model of social phobia. The model comprises 5 components, which build upon the fundamental notion that people with SAD assume that others are overly critical of them, and judge them negatively. They also attribute a great importance to being evaluated positively by others (Rapee and Heimberg, 1997).
The first component entails forming a mental representation of how the audience (i.e. any one person or group of people present) presumably perceives the individual's external appearance and behaviour. The second component is attentional resource allocation, meaning that the individual will rapidly turn his or her attention to the detection of threat. However, at the same time, the individual must monitor the mental representation

of the self and in particular those aspects of the individual's external appearance or behaviour that can elicit negative evaluation. The third component is formed by a prediction of the expected performance standard set by the audience, which is based on the characteristics of the situation and the audience (i.e. is it a formal or informal situation, does the audience consist of people that are more important or less important than the individual). This, in turn, will determine the perceived likelihood of negative evaluation. The fourth component is anxiety. This anxiety follows from the predicted negative evaluation and has physiological, cognitive and behavioural aspects. These aspects influence the individual's mental representation of his or her own external appearance or behaviour as seen by the audience, which renews the cycle starting with component one (Rapee and Heimberg, 1997).

Representation of the self and the external world (i.e. the audience) is evidently the central focus of this model. Although not explicitly stated by Rapee and Heimberg (1997), it is very plausible that creating a mental representation of how one is perceived by an audience relies heavily on information derived from facial expressions from the audience. In support of this, findings of functional imaging studies in SAD have been fairly consistent in reporting that individuals with SAD demonstrate increased amygdala and frontal-striatal cortices activity when exposed to negative or threatening facial expressions (Fouche *et al.*, 2013). Additionally, heightened self-referential processing has also been shown (Blair *et al.*, 2011b), and representation of the self is an important aspect of Rapee and Heimberg's model. However, it should be noted that these are interpretations that have not been incorporated in a neuroanatomical model on SAD.

Taken together, in line with other current literature on functional and structural connectivity studies, our results point to the involvement of widespread brain regions in SAD, including limbic, temporal, frontal, parietal and occipital areas. The recent growth in number of neuroimaging studies (particularly connectivity) may be an indicator that a neuroanatomical model of SAD may be developed in the near future.

Generalised anxiety disorder

Whereas the unique contributions of PD and SAD were discussed in Chapters 2 and 3 respectively, GAD was not separately addressed in this

thesis. The NESDA sample, from which the participants were drawn for Chapters 2, 3, and 4, did not include participants with GAD alone, preventing us from addressing this explicitly in our research. We have therefore not been able to contribute to the literature on GAD alone.

It is of note that GAD is distinctly understudied, despite its frequent comorbidity with depression and other anxiety disorders (Kessler *et al.*, 2005b). In addition, where other anxiety disorders and depression have been contrasted to one another, this has only been done once for GAD, making it difficult to outline the similarities and differences with related disorders (Hilbert *et al.*, 2014). However, based on the small body of available literature on the neurobiology, Hilbert and colleagues (2014) propose a tentative neurobiological model of GAD in their very recent review. The authors suggest that in GAD patients, apprehensive expectation leads to amygdala hyperactivation, which in turn leads to elevated cortisol levels. Due to disturbed emotion regulation by the vlPFC and ACC, cognitive top-down control in the form of worrying as a coping mechanism is ineffective, leading to uncontrollable worries as well as chronic cortisol secretion. As a result, serotonin uptake reaches a maximum and causes the affective changes in GAD patients. Additionally, an increased cortisol level decreases functional connectivity between the amygdala and the PFC, also affecting emotion regulation and increasing anxiety (Hilbert *et al.*, 2014). Whereas the authors feel that their neurobiological model matches contemporary models and theories in other modalities (e.g. psychology), they acknowledge that further work is needed to substantiate and expand this model. However, this newly proposed neurobiological model of GAD could be a fitting and relevant framework that can be used as a reference for future studies.

Depression

Helen Mayberg proposed a pioneering neurobiological model of depression with a focus on disruption of a widely distributed and functionally interactive network of cortico-limbic pathways (Mayberg, 1997). In her model, three compartments (dorsal, ventral, and rostral) are held responsible for maintaining an intact mental state, and dysfunction of either element as well as dysregulated collaboration between them is hypothesised to underlie depressive symptomatology. The inhibition of hyperactive ventral areas and restored activity of hypoactive dorsal regions

is proposed to result in remission (Mayberg, 1997). By offering the model as an adaptable framework, Mayberg invited researchers from various fields to integrate their findings. Indeed, depression has attracted much attention over the past decades with a growing amount of neuroimaging research. Subsequent studies contributed to the development of a model of emotion perception by Phillips and colleagues, with an interpretation for depression pointing to the involvement of a ventral and a dorsal system (Phillips *et al.*, 2003a, Phillips *et al.*, 2003b). For a normal emotional perception, the ventral system is responsible for identifying the emotional significance of environmental stimuli and for the production of affective states. This system is proposed to be disturbed in depression. Similarly, the dorsal system is involved in higher cognitive processes such as planning and selective attention, and contributes to a more effortful regulation of the affective state. Phillips *et al.* advocate that these processes are biased by emotional input in depression (Phillips *et al.*, 2003a, Phillips *et al.*, 2003b).

Providing support for Mayberg's and Phillips' theories, many studies have investigated the roles of separate brain regions in anatomical and functional studies, with findings consistently highlighting abnormalities of the hypothalamus, pituitary, hippocampus, amygdala, and prefrontal regions as the ACC, dorsolateral and orbitofrontal cortex (Hulvershorn *et al.*, 2011). Particularly the role of anterior cingulate regions has received a great deal of attention. A meta-analysis by Pizzagalli (2011) revealed that pre-treatment elevated rostral – also known as pregenual – ACC resting cerebral blood flow is a reliable predictor of better treatment response in depression (Pizzagalli, 2011). This was originally reported by Mayberg herself in a PET study in hospitalised depressed subjects, where hypometabolism of the pregenual ACC marked non-responders to treatment (Mayberg *et al.*, 1997). Subsequent studies confirmed this finding and also described the link between better treatment response and hyperactivity in subgenual ACC regions (Mayberg, 2003) as well as hypoactivity in dACC regions (Davidson *et al.*, 2002, Pizzagalli, 2011).
Aberrancies of the anterior cingulate also emulate from structural studies, with reports of reduced grey matter volume in depressed subjects compared to healthy controls (Koolschijn *et al.*, 2009). In contrast, there are also reports of no dACC volume differences between groups (Pizzagalli, 2011). However, our finding of reduced dACC grey matter volume in clinically

depressed adolescents (Pannekoek *et al.*, 2014b) confirms findings from the majority of structural studies in depressed subjects. This further substantiates the neurobiological models emphasising a role of the ACC in depression. It is still unknown whether abnormalities of this brain region precede or follow the onset of MDD. However, our findings stem from a study in treatment-naïve depressed adolescents that were included straight after first referral to a clinician. This suggests that our participants were in an early stage of the disorder, which could indicate that the smaller dACC grey matter volume was present before disease onset. Nevertheless, we stress that future studies should confirm this speculation. Our resting-state study in the same adolescent sample also showed abnormalities of the ACC in depressed subjects. Confirming previous literature as well as Mayberg's model and Phillips' theory on emotion perception (Mayberg, 1997, Phillips *et al.*, 2003b, Etkin *et al.*, 2011a), we reported decreased resting-state functional connectivity between the amygdala and the pregenual ACC (Pannekoek *et al.*, 2014a). This provides further support for disturbances in the ventral system in which such deviations are proposed to underlie depressive symptomatology by both models (Mayberg, 1997, Phillips *et al.*, 2003b). Additionally, we reported decreased dACC connectivity with prefrontal regions, indicating that the dorsal system is also affected in depression. Mayberg depicts connections between dorsal cortical regions and prefrontal regions, suggesting that remission will occur when hypoactivity in the dorsal areas is restored (Mayberg, 1997).

All in all, the results of our studies are in favour of two important neurobiological models on depression (Mayberg, 1997) and emotion perception (Phillips *et al.*, 2003a, Phillips *et al.*, 2003b). It is plausible that frontocingulate dysfunction contributes to key cognitive and affective abnormalities in depression, such as rumination, a tendency to engage in and enhance negative information, and emotion dysregulation (Pizzagalli, 2011).

Comorbid depression and anxiety

Investigation of comorbid depression and anxiety can have important treatment implications. With the topic of the shared or separate etiology of depression and anxiety disorders still hotly debated (Ressler and Mayberg, 2007), the available literature is not yet able to provide a definitive answer.

The results from Chapter 4 of this thesis, reporting altered RSFC in a limbic network in comorbid depressed and anxious adults compared to healthy controls, but no differences in depression or anxiety alone, suggested that the effect is specific to comorbid depression and anxiety, only becoming evident when patients present with both disorders and not in patients with a single diagnosis of either. No correlations with symptom severity were found. Whereas comorbidity is associated with higher severity (Kessler *et al.*, 2012), tailored treatments for anxiety disorders can prove as effective for individuals with and without comorbid depressive and anxiety disorders, and significantly reduce symptoms (Allen *et al.*, 2010).

Developmental aspects

Rapid and dramatic developmental changes, for example in hormonal and neural systems, are characteristic for the pubertal years. Adolescence is therefore regarded as a particularly sensitive period for vulnerability for and the development of psychopathology. Indeed, many psychiatric illnesses, including mood and anxiety disorders, most commonly have their origin during adolescence and are likely to be associated with abnormalities of typical maturational processes. The onset of affective disorders in youngsters is not only related to a greater disease severity, but anxiety symptoms frequently precede depression in adolescence (Paus *et al.*, 2008). In turn, adolescent onset of depression is a predictor for other mental health disorders in adulthood, such as anxiety disorders, substance-related disorders and suicidal behaviour (Thapar *et al.*, 2012). Because of these far-reaching and even lifelong implications and the paucity of early interventions and treatments to address these implications, more insight into the underlying normal and abnormal developmental trajectories is warranted (Paus *et al.*, 2008). In Chapters 5 and 6 we demonstrated functional and structural abnormalities in clinically depressed adolescents that were included in the study immediately after referral by their clinician, suggesting that these aberrancies may have been present prior to the development of psychopathology. However, causality could not be assessed in the current study design. Our results are nevertheless a valuable contribution to the research field of adolescent affective disorders. Neuroimaging studies on the typically developing brain are essential to construct a template that can serve as a reference for the abnormally developing brain. In addition, integrating multiple imaging modalities

with other factors (e.g. genetic, psychosocial and environmental) in a longitudinal design will provide a more comprehensive view on the various contributors to the development of affective disorders (Paus *et al*., 2008).

Clinical implications

The current diagnostic system for mental disorders is based on a categorical approach that relies on the presence of absence of certain symptoms (Brown and Barlow, 2005, Krishnan and Nestler, 2008). A DSM diagnosis of depression requires the presence of a certain number of symptoms that significantly impair functioning for a specific duration. As a result, two patients with the same diagnosis can actually only have one symptom in common (Krishnan and Nestler, 2008). On the other hand, the symptoms can overlap between different disorders (such as depression and anxiety) that are thought to have a different etiology (Krishnan and Nestler, 2008). The ongoing debate about the advantages and disadvantages of a dimensional diagnostic approach instead of or in addition to a categorical approach is beyond the scope of this thesis. However, these variations within one disorder and overlap of symptoms with other disorders signify how challenging it is to offer a straightforward interpretation of study results (Krishnan and Nestler, 2008).

Considerations and limitations

NESDA

The NESDA neuroimaging study is embedded in the large longitudinal framework of the general NESDA study. This unique design offers the opportunity to monitor a large, well-characterised sample presenting with an extensive variety of symptoms over a long period: multiple assessment waves have been done since its commencement in 2004 and the study is still ongoing. Despite the advantages of such a design, some considerations are important to note.

Patients for the NESDA sample were recruited through general practitioners, primary care practices, and outpatient clinics of mental health organisations. It is therefore possible that the most severely affected patients are not included. On a related note, it is probable that patients with the severest symptomatology are not motivated to partake in the extensive initial assessment, with a consecutive two-hour MRI scanning session and interview for the neuroimaging study.

Some issues concerning the imaging component of NESDA should also be considered. The resting-state scan was acquired at the end of the imaging protocol: after completion of three task-related functional MRI runs and the acquisition of an anatomical scan (scan sequence: Tower of London, word encoding, T1-weighted scan, word recognition, perception of facial expression, resting-state). It is therefore possible that the task-related functional MRI session preceding the resting-state fMRI run have influenced RSFC. Positioning the resting-state fMRI run at the beginning of a scanning protocol could help prevent these so-called spillover effects, which is something that would be recommended for future studies. Also, the current design did not include a DTI run. With the current increased emphasis on connectivity in (neuro)psychiatric imaging studies, including DTI in the NESDA neuroimaging protocol would have resulted in a considerable amount of cross-sectional and longitudinal data, and thus provided a valuable contribution to contemporary mental health research. In line with this, it would also be of interest to investigate whether abnormalities in functional connectivity are accompanied by abnormalities in structural connectivity, and whether they are associated or even predictive.

EPISCA

The EPISCA study captures a representative cohort of adolescents with internalising problems by including youngsters with depressive and anxious symptomatology, as well as a group of adolescents with a history of sexual trauma (the latter is not discussed in this thesis). Because the participants were included before starting any type of treatment and were scanned twice more with periods of three months in between, this longitudinal study design is suited for the assessment of treatment effects. The resting-state run was the first run of the scanning protocol, thus preventing the potential influence of task-induced activity on the RSFC.

It is of note that the EPISCA sample is relatively small, and larger groups would be desirable in future studies. Another consideration for future research would be to explicitly define illness duration. The adolescents were recruited after referral by a clinician and before treatment onset, suggesting that they were in an early stage of the disease. Nevertheless, it would be beneficial to assess the exact duration of the complaints. Also, the male/female distribution was very unequal: only

three males were included versus 23 females in the two EPISCA studies in this thesis. Gender comparisons were therefore impossible. However, as females are more likely to develop depression (Kessler *et al.*, 2005b), the EPISCA sample is representative of the general population. Finally, the resting-state fMRI run had a duration of 6 minutes. Whereas it was standard in studies to use 5–7 minutes of resting-state data, it has been shown that reliability of RSFC improves significantly with an increase in scan duration as well as in the number of volumes (Birn *et al.*, 2013).

Resting-state

A big advantage of resting-state fMRI is that it offers an unbiased approach to investigate functional connectivity of the brain. This is of especially great importance in clinical groups, where persons suffering from, for example, neurodegenerative or (neuro)psychiatric disorders are less capable or even incapable of dealing with the cognitive demand that functional tasks often entail. However, interpretation of RSFC results is not necessarily straightforward. Functional connections between brain regions are correlations between timeseries of particular nodes (or regions of interest), and do not imply causality or directionality (i.e. is the connectivity between two nodes direct, or is it mediated by a third). These correlations are therefore also more susceptible to being influenced by confounders, such as noise and artefacts (Smith, 2012).

In addition to head motion, cardiac and respiratory fluctuations are examples of physiological noise that can have an effect on RSFC, and ways to eliminate them are constantly improved(Birn, 2012). Physiological activity was not monitored during scanning in the studies discussed in this thesis. However, heart rate variability was investigated in the entire NESDA sample, and showed that heart rate variability was lower in depressed subjects (Licht *et al.*, 2008) as well as in anxious subjects (Licht *et al.*, 2009) compared to healthy controls. It is important to note that in both these studies the lower heart rate variability in the patient groups appeared to be driven by antidepressant use (Licht *et al.*, 2008, Licht *et al.*, 2009). In our studies, using subsamples of the NESDA neuroimaging study, we only included medication-free and largely even medication-naïve subjects. Also, it has been shown that independent component analysis (applied in Chapter 4) can detect signal sources associated with confounding physiological activity and that it can distinguish these from signals of

interest (Beckmann *et al.*, 2005). We therefore think that it is unlikely that the differences in Chapter 4 were introduced by these physiological signals. Additionally, in our studies using a seed-based region-of-interest approach (Chapters 2, 3 and 5 (Pannekoek *et al.*, 2013b, Pannekoek *et al.*, 2013c, Pannekoek *et al.*, 2014a)), the global signal was regressed out to reduce artefacts as a result of physiological signal sources, in accordance with previous studies indicating that such a procedure is a successful tool for this purpose (Birn *et al.*, 2006, Fox and Raichle, 2007, Birn, 2012).

A topic of some controversy concerning resting-state fMRI is the existence of negative functional connectivity. This refers to negative correlations between the timeseries of two brain regions, and is currently still poorly understood. It has been argued that these correlations are introduced by global signal regression during data preprocessing (Murphy *et al.*, 2009, Cole *et al.*, 2010, Chen *et al.*, 2011), suggesting that this method may have some disadvantages. However, global signal regression is also a powerful and important tool to remove motion artefacts in resting-state fMRI (Power *et al.*, 2014, Yan *et al.*, 2013) and as discussed above, as well as physiological noise. In addition, it has been shown that the negative connectivity exists with or without correction for global signal (Chang and Glover, 2009). At present, as reports have been equivocal, the precise meaning of negative connectivity remains open to debate (Cole *et al.*, 2010).

It is clear that like other MRI modalities, resting-state fMRI has its advantages as well as limitations. As one of the newest imaging approaches, it is in constant development and the research techniques are continuously improved. Experts in the field of functional connectivity point out the links that are already made between changes in connectivity and diseases, and predict an even further growth in its clinical applications. They are confident that in the future, functional connectivity has the potential to become a powerful instrument for investigating disease mechanisms, particularly when combined with other imaging modalities including structural MRI and diffusion-based connectivity, but also non-MRI modalities such as magnetoencephalography and electroencephalography (Smith, 2012).

Future directions

Structural and functional magnetic resonance imaging have provided important insights into the involvement of candidate brain regions. It is unlikely that simple increases and decreases in brain activity alone are sufficient to explain the complex diversity of symptoms that define depression and anxiety (Krishnan and Nestler, 2008). Combined efforts using various research modalities (for example, MRI, (epi)genetics, neuroendocrinology, and environmental studies) could significantly improve knowledge about the neurobiology and pathophysiology of depression and anxiety disorders. Performing such complementing multi-modal studies, and thus acknowledging and embracing the heterogenetic nature of these disorders, would be a promising next phase of neurobiological research on affective disorders. This multivariate approach is a critical next step in the eventual development of diagnostic guidelines to distinguish patient subgroups, map comorbidity and the extent of its influence on disease course and treatment outcome, construct optimal intervention strategies, predict the disease course and identify biomarkers for vulnerability.