Summary and general discussion
Summary of main findings

Major depressive disorder (MDD) is a common and debilitating neuropsychiatric disorder. Stress is an important risk factor for the onset (Kendler et al., 1999; Daley et al., 2000), severity (Hammen et al., 1992) and course of MDD (Kendler et al. 1997). There is some evidence that the physiological response to stress is altered in MDD patients, as hyperactivity of the HPA-axis, increased oxidative stress and inflammation have been observed in MDD patients and have been implicated in the etiology of the disorder (Dean and Keshavan, 2017; Wigner et al., 2017). Dysregulation of these biological stress systems may contribute to the onset, severity and chronicity of MDD by increasing neurodegeneration and decreasing neurogenesis in brain regions involved in emotion-regulation of patients. As it is not possible to assess markers of these biological systems in the brain in vivo, research has focused on these markers in peripheral blood. As it remained unclear how peripheral markers of these biological stress systems were related to brain morphology in humans, the first part of this thesis was dedicated to investigating how oxidative stress, inflammation, and related metabolic dysregulation are associated with neuroimaging measures of regional brain morphology. Furthermore, the association between an important environmental risk factor for MDD, childhood maltreatment, and brain morphology was examined and we examined whether this association was related to an essential protein for neurogenesis, brain-derived neurotrophic factor (BDNF). In the second part of this thesis, neuroimaging methods were used to study differences in brain structure and function between MDD patients and healthy controls.

Part I: Biological stress systems and the brain

Chapter 2 focused on the question whether childhood maltreatment (CM) and BDNF were associated with brain morphology. The main effects of CM and different markers in the BDNF pathway (at the gene, gene expression and protein level), and the interaction between CM and BDNF on volume of the hippocampus and amygdala and cortical thickness and surface area of the anterior cingulate cortex (ACC) were examined. To this end, repeated measures ANOVA analyses were performed, in which hemisphere (left or right) was added to the model as a within-group factor, corrected for differences in age, sex, education level, scan site, intracranial volume, and presence of depression and/or an anxiety disorder. In secondary analyses we examined whether the presence of an affective disorder had a similar effect on brain morphology as CM. In our NESDA imaging study, 146 participants had a history of CM, while 143 participants did not.

Our findings revealed that CM was associated with decreased amygdala volume (p=0.038; N=289), while BDNF was not directly associated with brain morphology at any level (see
overview in Table 1). There was a significant interaction effect between CM and Val66Met genotype on amygdala volume ($p<.001; N=255$), suggesting that the effect of CM on amygdala volume was stronger in carriers of the met-allele. An association between CM and BDNF was also observed at the gene expression level ($p=0.010; N=195$), and this effect appeared to be independent from the association with BDNF genotype in post-hoc analyses.

Right rostral ACC thickness was lower in maltreated individuals with a val/val genotype, showing that CM has different effects on brain morphology in met-carriers and val-homozygotes.

**Chapter 3** examined how immunometabolic dysregulation, which consists of low-grade inflammation and metabolic dysregulation, was associated with hippocampal and amygdala volume and ACC thickness using linear regression analyses. To this end, associations with inflammation markers (tumor-necrosis factor-alpha (TNF-α), c-reactive protein (CRP) and interleukin-6 (IL-6)), BMI, triglyceride levels and HDL-cholesterol and brain morphology were examined. Furthermore, the associations between polygenic risk scores for three of these markers and brain morphology were investigated. In secondary analyses, we examined whether these associations were similar in patients with depression and/or anxiety and healthy controls, and performed repeated measures analyses to examine whether these effects were driven by the left or right hemisphere. Findings reveal that multiple serum, but not genetic markers of immunometabolic dysregulation were negatively associated with rostral ACC thickness (BMI $p=0.021$; waist circumference: $p=0.016$; triglyceride levels: $p=0.006$; CRP: $p=0.045$; TNFa: $p=0.018$; $N=283$) and these associations were similar in patients and healthy controls, suggesting that these markers may impact the ACC in patients and controls through similar mechanisms.

**Chapter 4** focused on the association between two measures of oxidative stress (8OHdG and F2-isoprostanes) and volume of the amygdala, hippocampus and hippocampal subfields, which were assessed using linear regression analyses ($N=297$). Secondary analyses examined if these associations are similar in healthy controls and patients with an affective disorder. There were no significant associations between oxidative stress markers and brain morphology, nor was there a significant interaction effect between oxidative stress markers and presence of an affective disorder on brain morphology (all $p>.05$).
Table 1. Main results of chapter 2-4 of this thesis

ACC: anterior cingulate cortex; N.E: not examined; *: in interaction with; #: does not survive correction for multiple testing

<table>
<thead>
<tr>
<th></th>
<th>Hippocampus volume</th>
<th>Hippocampal subfields volume</th>
<th>Amygdala Volume</th>
<th>ACC thickness</th>
<th>ACC surface area</th>
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<tbody>
<tr>
<td><strong>Childhood maltreatment, BDNF &amp; brain morphology (chapter 2)</strong></td>
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<tr>
<td>Main effect CM</td>
<td>No association</td>
<td>N.E.</td>
<td>Lower amygdala volume in maltreated participants</td>
<td>No association</td>
<td>No association</td>
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<tr>
<td>Main effect BDNF</td>
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<td>N.E.</td>
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<tr>
<td>CM*BDNF genotype</td>
<td>No association</td>
<td>N.E.</td>
<td>Lower amygdala volume in maltreated met-allele carriers</td>
<td>Lower rostral ACC thickness in maltreated val-homozygotes</td>
<td>No association</td>
</tr>
<tr>
<td>CM*BDNF gene expression</td>
<td>No association</td>
<td>N.E.</td>
<td>Positive association with BDNF gene expression, only in non-maltreated subjects</td>
<td>Trend for positive association with BDNF expression in non-maltreated subjects only</td>
<td>No association</td>
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<tr>
<td>CM*BDNF protein levels</td>
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<td>No association</td>
<td>No association</td>
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<tr>
<td><strong>Immunometabolic dysregulation &amp; brain morphology (chapter 3)</strong></td>
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<td>Inflammation markers (IL-6, TNFa, CRP)</td>
<td>IL-6 negatively associated with hippocampal volume$^a$</td>
<td>N.E.</td>
<td>No association</td>
<td>CRP &amp; TNF-a were negatively associated with rostral ACC thickness$^a$</td>
<td>N.E.</td>
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<tr>
<td>Metabolic markers (BMI, triglyceride levels, HDL-cholesterol)</td>
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<td>N.E.</td>
<td>No association</td>
<td>BMI*, waist circumference* and triglyceride level were negatively associated with rostral ACC thickness</td>
<td>N.E.</td>
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<tr>
<td>Genetic markers</td>
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<td>No association</td>
<td>No association</td>
<td>N.E.</td>
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<td>inflammation/metabolic dysregulation</td>
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<td>Oxidative stress &amp; brain morphology (chapter 4)</td>
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<td>8OHdG</td>
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<td>F2-isoprostanes</td>
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<td>8-OHdG * diagnosis</td>
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<td>No association</td>
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<td>F2-isoprostanes*diagnosis</td>
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Part II: Neuroimaging of Major Depressive Disorder

In chapter 5 a coordinated approach was used to perform a prospective meta-analysis of diffusion tensor imaging (DTI) studies within the Major Depressive Disorder Working Group of the ENIGMA (Enhancing Neuroimaging Genetics through Meta-Analysis) consortium. In this largest individual participant data based meta-analysis to date, DTI data from 20 sites worldwide were included, resulting in a total of scans from 1305 MDD patients and 1602 healthy controls. The aim was to examine differences in white matter (WM) anisotropy and diffusivity and to examine associations between these measures and clinical characteristics of MDD. Harmonized protocols were used for processing of DTI data at each site. Statistical analysis was also harmonized across sites and effects were meta-analyzed across studies. Findings reveal widespread, but subtle, reduction of fractional anisotropy (FA) and increased mean and radial diffusivity (MD and RD) in adult patients. Strongest regional effects were observed in the corpus callosum and corona radiata. These findings were driven by patients with recurrent MDD and an adult age of onset. While lower FA of the corpus callosum was also seen in adolescent MDD patients (under 22 years of age), these findings did not survive correction for multiple testing.

Chapter 6 focused on the association between cumulative disease load of depression and anxiety psychopathology and network characteristics of structural and functional brain networks in MDD patients (N=77). Disease load was determined by calculating the percentage of time over nine years with depression and/or anxiety symptoms, and was determined on basis of detailed clinical information collected at five interviews over the nine years preceding the neuroimaging session. Graph theoretical analysis of resting-state functional MRI (rsfMRI) and DTI data was then performed to examine network characteristics of functional and structural brain networks. Our findings show that disease load had a differential effect on structural and functional brain network properties. In structural networks, a loss of efficient small-world characteristics was observed, while in functional networks increased functional connectivity was seen in regions involved in the default mode network.

General discussion

Part I: Biological stress markers and brain morphology

Aim I: Investigate the relationship between childhood maltreatment, BDNF and brain morphology
Childhood maltreatment, defined as neglect, physical abuse and/or sexual abuse before 18 years of age, is an important risk factor for the development of psychiatric disorders (Jaffee et al., 2017). This form of early life stress takes place at a time when the developing brain is vulnerable to the effects of stress hormones (Lupien et al., 2009). Chapter 2 of this thesis examined how a history of childhood maltreatment and BDNF, an essential protein involved in neurogenesis and neuroplasticity, were related to brain morphology. Previous studies have shown a gene-by-environment interaction on brain volume, where the effect of childhood trauma on brain structure was stronger in carriers of the met-allele of the Val66Met SNP in the BDNF gene. This polymorphism is a functionally relevant SNP of which the met-allele has been associated with lower BDNF secretion (Egan et al., 2003). It therefore appears that the effect of childhood trauma on brain structure is more prominent in individuals with a biological vulnerability (Gerritsen et al., 2012; Frodl et al., 2014; Gatt et al., 2009). These previous studies only focused on BDNF Val66Met genotype, therefore we examined the associations between CM, BDNF at the genotype, gene expression and protein level, and brain morphology.

Findings of this study show that CM was associated with lower amygdala volume and that this was especially evident in carriers of a met-allele. Previous studies have linked childhood maltreatment to both increased amygdala volume (Pechtel et al., 2014), and decreased amygdala volume (Edminston et al., 2011; Hanson et al., 2015; Lim et al., 2014), whereas other studies found no association with amygdala volume (Woon and Hedges, 2008). It has been suggested that amygdala volume increases following maltreatment and then decreases over time (Hanson et al., 2014). This variation over time may explain different findings across studies and may also explain why a recent meta-analysis of the existing literature failed to observe an association between maltreatment and amygdala volume (Calem et al., 2017). The findings also suggested that lower amygdala volume may be more prominent in carriers of the met-allele. We speculated that decreased amygdala volume in maltreated met-carriers might be associated with emotion regulation impairment in maltreated individuals. In support of this, another study observed an interaction effect between childhood maltreatment and BDNF genotype on emotion regulation, with impaired emotion regulation being observed in maltreated carriers of the met-allele (Miu et al., 2017), the group that also showed lowest amygdala volume in our study.

Lower rostral anterior cingulate cortex (ACC) thickness was observed in maltreated subjects who were homozygous for the val-allele. The rostral ACC, which in this study includes the pregenual ACC and a part of the subgenual ACC, is a part of the medial prefrontal cortex and is important for multiple cognitive and affective processes, including emotion regulation (Stevens et al., 2011). While the met-allele is usually regarded as the risk-allele, there is some recent evidence that suggests that individuals with a val/val genotype show an increased cortisol response and increased depressive symptoms to mental stress (Jiang et al., 2017;
Alexander et al., 2010). With respect to the existing literature, this association was still unexpected and requires replication in cross-sectional studies and in experimental studies in animal models to better understand the underlying mechanisms.

Interestingly, maltreatment-by-BDNF interaction effects were observed for ACC thickness, but not surface area. Both surface area and cortical thickness contribute to brain volume, but result from different neurobiological processes, have different developmental trajectories and are regulated by different genes (Panizzon et al., 2009). Our findings are in line with previous studies that showed an effect of maltreatment on cortical thickness, but not cortical surface area (Lim et al., 2017). A study from the MDD working group of the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) consortium, which included neuroimaging data from 3106 participants, concluded that maltreated individuals showed an accelerated reduction in cortical thickness, but not surface area, with age (Frodl et al., submitted), which also showed that cortical thickness, not surface area is vulnerable to stress.

In post-hoc analyses we examined the effect of the presence of an affective disorder in the six months preceding the scan, and in interaction with BDNF, on brain morphology. Results from the interaction analyses suggest that all associations with amygdala volume are independent of MDD diagnosis, but that findings with rostral ACC thickness may be at least partly related to MDD. In analyses performed after publication of the study, we examined three-way interaction effects between maltreatment, BDNF and presence of an affective disorder on brain morphology and did not find significant results, suggesting that the interaction effects between CM and BDNF on brain structure, are not driven by clinical status.

In our study, no association with hippocampal volume was observed. Previous work has inconsistently associated hippocampal volume with maltreatment (Lim et al., 2014; Calem et al., 2017), BDNF Val66Met genotype (Molendijk et al., 2012, Harrisberger et al., 2015) and BDNF Val66Met genotype-by-maltreatment effects have also been observed on hippocampal volume, albeit again inconsistently (Aas et al., 2013; Gerritsen et al., 2012). While initial studies observed strong associations between BDNF and hippocampal volume, replication has been problematic and these studies may have been underpowered and their results inflated due to publication bias (Molendijk et al., 2012). Overall, there is little evidence to suggest an association between BDNF and childhood maltreatment with hippocampal volume based on the existing literature.

Significant associations between CM and BDNF on brain morphology were observed at both the gene and gene expression level, but not at the serum protein level. Post-hoc analyses revealed that the genotype and gene expression results were independent findings, suggesting that the interaction effect of gene expression and CM on amygdala volume and rostral ACC thickness were not related to genotype. Previous studies did not find an association between BDNF serum levels and BDNF Val66Met genotype (Luykx et al., 2013;
Skibinska et al., 2017). In line with this, no significant associations were observed between BDNF genotype, gene expression levels and serum protein levels. BDNF serum levels may vary more over time and be influenced by other factors, including sampling factors, sociodemographic and lifestyle factors and biological factors such as cytokines, glucocorticoids or sex hormones, which may obscure an association with brain morphology and childhood trauma (Bus et al., 2011). Furthermore, it remains unclear if peripheral BDNF reflects BDNF levels in the brain (see discussion below). Noise related to determination of BDNF in blood may explain why we did not find associations with serum protein levels.

Studying interactions between biological and environmental factors such as childhood maltreatment is important, as it may help identify biopsychosocial subgroups, which may be at risk for psychopathology. Increasing our understanding of the mechanisms or pathways underlying this risk may lead to more individualized prevention and treatment schemes. Findings from this study suggests that that there may be different BDNF-related subtypes that are important for the effect of childhood trauma on brain morphology.

**Aim II: Biological stress markers & brain morphology**

Chapter 3 and 4 of this thesis investigated how markers of the biological stress systems were related to brain morphology. This is important as it may reveal potential pathways through which biological stress may increase vulnerability for depression and anxiety symptoms. Chapter 3 focused on immunometabolic dysregulation, which consists of low-grade inflammation and metabolic dysregulation. These two systems are closely linked, as adipose cells are an important source of pro-inflammatory cytokines and they may share similar genetic regulation (Kraja et al., 2014 & Debnath et al., 2016). We observed that multiple markers of immunometabolic dysregulation were negatively associated with rostral ACC thickness (e.g. BMI, waist circumference, triglyceride levels, CRP, TNF-a and a composite measure of immunometabolic dysregulation), but that only findings with triglyceride level and an overall measure of immunometabolic dysregulation survived correction for multiple testing. The rostral ACC, which is important for both processing of emotion and the cognitive

**CONCLUSIONS:**

1. **CHILDHOOD MALTREATMENT HAS A DIFFERENTIAL EFFECT ON BRAIN MORPHOLOGY IN DIFFERENT BDNF BASED SUBTYPES**
2. **MALTREATED MET-ALLELE CARRIERS MIGHT BE AT RISK FOR DEVELOPMENT OF PSYCHOPATHOLOGY THROUGH LOWER AMYGDALA VOLUME AND EMOTION REGULATION DEFICITS**
3. **IDENTIFYING MECHANISMS THROUGH WHICH MALTREATMENT AFFECTS THE BRAIN MAY LEAD TO NEW OPTIONS FOR PREVENTION AND PERSONALIZED TREATMENT**
aspects of emotion regulation (Szekely et al., 2017; Bush et al., 2000), may be particularly sensitive to peripheral inflammation and metabolic dysregulation. In line with this, multiple studies have shown a positive association between peripheral cytokines and ACC activity and connectivity (Slavich et al., 2010; Harrison et al., 2009; Marsland et al., 2017). While the precise cellular mechanisms underlying this vulnerability remain unclear, rodent studies have found that the ACC is vulnerable to stress, due to high expression of glucocorticoid receptors (GR), but not mineralcorticoid receptors (MR) (Cerqueira et al., 2005). Selective regional neuronal vulnerability to biological stress is complex and may be related to various factors including regional gene expression profiles (Wang & Michaelis, 2010), regional metabolism and activity, presence of neurotrophic factors/antioxidants and glutamate activity.

Interestingly, post-hoc tests revealed that IL-6 levels were negatively associated with hippocampal and amygdala volume in healthy controls, but not in patients. As IL-6 levels did not differ between patients and controls, it is possible that psychopathology-related changes downstream of the receptor or downregulation of the receptor due to chronic inflammation, obscure this association in patients. A second explanation may be that a large group of patients used antidepressants, which are known for their neurotrophic properties. We speculated that these neurotrophic effects may prevent or counteract damage to the rostral ACC in patients. However, additional analyses performed after publication, revealed that the absence of a negative association between IL-6 and brain volume in patients was not due to the effect of antidepressant use (data not shown).

Chapter 4 focused on two markers of oxidative stress and their association with volume of the amygdala and hippocampus. While many experimental studies in animal models have revealed that the hippocampus (including the cornu ammonis (CA)-1 and 3 and dentate gyrus subregions) and amygdala are most vulnerable to oxidative stress (Chang et al., 2012; Huang et al., 2012; Uysal et al., 2012; Salim et al., 2017; Wang & Michaelis, 2010), the evidence for an association between oxidative stress and brain morphology in humans is less clear. To date, only one study examined the association between peripheral markers of oxidative stress and brain morphology. Lindqvist et al. (2014) calculated a ‘total net anti-oxidant score’ based on oxidant and antioxidant markers and reported a positive association with hippocampal volume, particularly CA3 & dentate gyrus volume. In our study, we did not observe an association between two oxidative stress markers and hippocampal volume, amygdala volume or volume of the hippocampal subfields. While the association between oxidative stress and neurodegeneration is clear in animal studies, it is possible that oxidative stress does not affect brain morphology or that measuring peripheral oxidative stress is not a reflection of central oxidative stress (see discussion in methodological considerations below).
Inflammation, BDNF and oxidative stress are interconnected. In the NESDA sample, 8-OHdG, a measure of oxidative stress, was positively correlated with inflammation markers CRP, IL-6 and TNF-a, while F2-isoprostanes was positively correlated with CRP, although both correlations were weak (Black et al., 2017). It was surprising that while markers of inflammation were negatively associated with rostral ACC thickness, and hippocampal and amygdala volume in healthy controls, oxidative stress and BDNF were not directly associated with brain morphology. While cortical thickness of the ACC was not included as a region of interest in chapter 4, additional analyses performed after publication revealed that oxidative stress was also not associated with rostral ACC thickness (data not shown). These results suggest that the association between immunometabolic dysregulation and brain morphology is not likely mediated by oxidative stress.

The abovementioned findings are summarized in Figure 1. In brief, environmental stress factors and genetic factors interact to increase biological stress, including an increase in oxidative stress, inflammation, HPA-axis activity and a decrease in BDNF (Mitoma et al., 2008; Marsland et al., 2017; Pagliaccio et al., 2014). These stress systems are all bi-directionally connected. Inflammation was associated with lower rostral ACC thickness, while BDNF interacted with childhood trauma to affect amygdala volume and rostral ACC thickness. Both lower amygdala volume and decreased rostral ACC thickness have been associated with depression and anxiety disorders (Hamilton et al., 2008; Milham et al., 2005; Schmaal et al., 2017; Frick et al., 2013), and we speculated that decreased thickness or volume of these regions may explain how inflammation and maltreatment are associated with pathophysiology of depression and anxiety.

![Figure 1. Summary of main findings. Environmental stress factors (represented with the lightning bolt) and genetic factors interact to cause biological stress, which affect amygdala volume and rostral ACC thickness, which in turn may affect the onset and course of affective disorders.](image-url)
However, there are several important points to consider. First of all, the association between affective disorders and inflammation, for instance, appears to be bidirectional. While longitudinal studies show that markers of inflammation IL-6 and CRP predict the onset of depressive symptoms, depression also predicts IL-6 and CRP levels at a later time point (Valkanova et al., 2013; Copeland et al., 2012). While biological stress may lead to neurodegeneration and cortical thinning in the rostral ACC, this region is also involved in sending feedback to the HPA-axis and decreased ACC volume may also cause impaired regulation of the HPA-axis, leading to a further increase in biological stress (MacLullich et al., 2006). Therefore we are unable to speculate on the direction of causality.

Secondly, in the studies in this thesis, there was limited evidence to suggest associations with the onset & course of affective disorders. We did not observe differences between patients and controls in markers of inflammation or oxidative stress. These effects may be subtle, as previous meta-analytic results suggest that oxidative stress (measured using 8-OHdG and F2-isoprostanes) is increased in MDD, with a small to moderate effect size (Hedge’s g of 0.31 and 0.48 for 8-OHdG and F2-isoprostanes respectively) (Black et al., 2015). In a larger sample from the NESDA study, however, associations between oxidative stress and depression and/or anxiety disorders ceased to be significant after correction for antidepressant use (Black et al., 2017). In this sample, the evidence for higher inflammation in depressed patients was also limited, as CRP was only slightly increased in male patients (Cohen’s d=0.21), and IL-6 and TNF-a did not differ between patients and controls (Vogelzangs et al., 2012). There is some evidence to suggest that inflammation may be more prominent in individuals with atypical symptoms of MDD (Lamers et al., 2013). Thus, the association between biological stress markers and affective disorders is subtle and may be affected by medication use, symptom profiles and lifestyle factors.

As expected, we observed a similar (negative) associations between biological stress and brain morphology in patients and controls, with the exception of IL-6 being associated with brain morphology in healthy controls only, which is described above. From the findings in this thesis, there is little evidence to suggest that changes in brain structure related to brain morphology are also related to the onset or course of affective disorders. However, our study did not have the longitudinal design needed to examine this in more detail. These longitudinal studies could be performed within the NESDA study. Within NESDA, immunometabolic dysregulation was found to predict the course of MDD (Vogelzangs et al., 2014), mediation analyses could be performed to examine whether this effect was mediated by brain morphology.

Thirdly, this model is overly simplistic, as biological stress systems may also influence function and connectivity of brain regions, not only volume or cortical morphology. To date, no studies have examined the association between oxidative stress and brain function/connectivity in humans, but several studies have shown associations between...
Finally, there are several other neurobiological mechanisms associated with affective disorders, which may also be associated with inflammation and oxidative stress. For instance, in response to inflammation, the production of tryptophan is altered, leading to an increase in oxidative stress and a decrease in serotonin levels (Berk et al., 2011). Lower serotonin levels have been implicated in the pathophysiology of affective disorders (Ressler et al., 2000). This shows that there are other pathways, besides brain morphology through which inflammation may impact depression or anxiety disorders. Cortisol levels and HPA-axis activity were also not investigated in this thesis and are strongly associated with inflammation, oxidative stress, brain morphology and affective disorders (Cerqueira et al., 2005; Lupien et al., 2018, Aschbacher et al., 2013). Finally, glutamate excitotoxicity may also play a role in the interplay between inflammation and affective disorders (Dantzer et al., 2014).

In two studies of this thesis (chapter 2 and 3) we examined associations between genetic factors related to biological stress and brain morphology. In chapter 2, we did not observe a direct effect of the BDNF Val66Met SNP on brain morphology, but only in interaction with childhood trauma. In chapter 3, we did not observe robust associations between polygenic risk scores (GPRS) for three biological stress markers and brain morphology. Caution is warranted when drawing conclusions from these studies, as the analysis with polygenic risk scores may have been underpowered (see discussion below), interaction analyses are vulnerable to spurious findings and replication is still needed. If the absence of a consistent association between GPRS and brain morphology was not related to low power, the results of these two studies suggest that other, non-genetic, factors may play an important role in the association between biological stress markers and brain structure.

In general, effect sizes in studies that have examined differences in biological stress markers between MDD patients and controls are small. Rostral ACC thickness, which has been associated with both immunometabolic dysregulation and MDD, may represent an intermediate phenotype of immunometabolic dysregulation. Brain morphology may be more closely related to MDD symptoms than peripheral inflammation, and therefore show stronger effects. Furthermore, neuroimaging markers related to brain morphology may be more stable over time, increasing reliability and clinical relevance. Neuroimaging biomarkers have also been used to differentiate between psychiatric disorders and have been shown to predict the course of MDD (Strakowski et al., 2002; Schmaal et al., 2015). However, the use of neuroimaging biomarkers for clinical purposes is hampered by the costs of neuroimaging, and this marker is not specific to MDD, as the rostral ACC has also been implicated in schizophrenia and bipolar disorder (Fornito et al., 2008; Abe et al., 2016). Furthermore, brain structure is determined by many cellular mechanisms and not only by immunometabolic dysregulation.
Part II: Brain structure and function in Major Depressive Disorder

Aim III: To examine alterations in structural and functional brain networks associated in depression and in relation to clinical characteristics of the disease

Chapter 5 and 6 investigated structural and functional neuroimaging correlates of MDD. Finding neuroimaging correlates of MDD is important as it increases our knowledge of the underlying pathophysiology, may reveal vulnerability factors and new targets for prevention, treatment or predicting the course of depression.

In chapter 5, a meta-analysis of studies on white matter abnormalities in depression was performed. Instead of a traditional meta-analysis based on existing literature, a prospective meta-analysis of DTI studies was performed within the MDD working group of the ENIGMA consortium. Harmonized protocols were used to process DTI scans and perform statistical analyses across 20 sites worldwide, with a total sample size of 1305 MDD patients and 1602 healthy controls. In this largest individual participant data based meta-analysis to date, subtle and widespread changes in overall fractional anisotropy (FA) and radial diffusivity (RD) were seen in adult patients, but not in adolescent patients. While the interpretation of anisotropy and diffusivity measures is complicated and histopathological validation is still needed, there is some evidence to suggest that lower FA reflects abnormal white matter integrity; while RD reflects myelin damage (Winklevski et al., 2018). While this was a widespread effect, which disappeared after correcting for average FA, the strongest regional effects were observed in the corona radiata and corpus callosum. Decreased FA in the corpus callosum of MDD patients is the most consistent finding in the literature, and is in line with previous meta-analysis of DTI studies in MDD (Liao et al., 2013; Wise et al., 2016; Chen et al., 2016). The corona radiata and corpus callosum connect regions that are important for mood regulation and may underlie symptoms related to mood regulation in patients (Pico-Perez et al., 2017). Interestingly, WM abnormalities were observed in adult MDD patients, but not in adolescent MDD patients. This may have been related to a smaller sample size in our analyses with adolescent MDD patients and controls, or WM abnormalities

Conclusions:

- Thickness of the rostral ACC may represent an intermediate phenotype of immunometabolic dysregulation, but lacks specificity and sensitivity to be used as a biomarker in a clinical setting
- Peripheral oxidative stress is not associated with brain morphology
- Longitudinal studies may reveal the direction of causality and whether changes in brain structure related to immunometabolic dysregulation increase vulnerability to affective disorders

• Thickness of the rostral ACC may represent an intermediate phenotype of immunometabolic dysregulation, but lacks specificity and sensitivity to be used as a biomarker in a clinical setting
• Peripheral oxidative stress is not associated with brain morphology
• Longitudinal studies may reveal the direction of causality and whether changes in brain structure related to immunometabolic dysregulation increase vulnerability to affective disorders
may have been less severe in adolescents due to lower duration and recurrence of MDD in adolescents compared to adults.

It remained unclear how brain structure and function are associated with disease load of affective disorders at the network level. Therefore, chapter 6 investigated the association between disease load and structural and functional brain networks, which were derived using graph theoretical analysis. Previous studies examined disease load as disease duration (by subtracting age of onset from current age) or as number of episodes, and both do not take into consideration the time spent without psychopathology, which is common in affective disorders due to the relapsing and remitting nature of the disorders. Furthermore, patients with chronic symptoms may have had only one episode, but with high disease load and long duration. In chapter 6, a clinical measure of disease load was created based on the presence of MDD and anxiety symptoms assessed at five time points across a nine-year period prior to the neuroimaging assessment. We then examined how this measure was associated with network characteristics of structural and functional brain networks assessed at the last time point. Results show that cumulative disease load had a differential effect on functional and structural networks. In structural networks, disease load was associated with loss of efficient small-world characteristics. In functional networks, we observed increased connectivity within the default-mode network.

In both chapters 5 and 6, WM tracts were examined, but using two different methods. In the meta-analysis, average WM anisotropy and diffusivity measures were examined across an entire WM tract using tract-based spatial statistics (TBSS). The analyses in chapter 6 focused on WM connectivity between all brain regions. Tractography was used to examine which brain regions were connected by WM tracts. Graph theory was then used to extract measures related to connectivity of these different brain regions. This second approach allows more detailed characterization of regional and whole-brain white matter connectivity and topology at the network level compared to TBSS. It would be interesting to use the graph theoretical approach in the ENIGMA MDD sample used in chapter 5, in order to perform a prospective, large-scale individual participant data based meta-analysis of studies similar to the study presented in chapter 5 in order to examine WM differences between patients and controls at a more detailed network level.

Results of the associations between disease load and network characteristics suggested that abnormalities in functional and structural networks increased with disease load or cumulative duration of symptoms, and that affective disorders may be neuroprogressive in nature. In line with this, the meta-analysis of DTI studies suggested that depression-related WM abnormalities in adults were driven by patients with recurrent MDD episodes, and were not observed in first-episode patients. It is clear from the findings in the first part of this thesis and from the existing literature that biological stress mechanisms (and in interaction with environmental stress factors) are associated with grey matter
morphology, but prolonged exposure to these stress factors may also impact white matter structure (Benedetti et al., 2016). Longitudinal studies may reveal whether changes in gray and white matter structure reflect the neurotoxic effect of prolonged symptoms of affective disorders or represent predictors of high disease load or recurrent depression.

There has been an interesting debate over the last years whether MDD should be considered a neuropsychiatric or brain disorder rather than a psychological or mental disorder. Proponents of defining MDD as a neuropsychiatric disorder argue that the beneficial effect of antidepressants, including SSRIs, suggests that a biochemical imbalance may underlie MDD and that therefore depression should be considered a neuropsychiatric disorder. Furthermore, they argue that MDD has been associated with abnormalities in brain morphology, function and connectivity in neuroimaging studies. Large-scale neuroimaging studies, including the study in chapter 5, suggest that differences between MDD patients and controls in WM, cortical and subcortical structure are subtle (Cohen’s d < 0.3) (Schmaal et al., 2016, Schmaal et al., 2017), in comparison to effect sizes in bipolar disorder (Hibar et al., 2016) and schizophrenia (Van Erp et al., 2016; Kelly et al., 2017). While bipolar disorder and schizophrenia are often considered traditional neuropsychiatric disorders, this is less the case for MDD. One reason for this is that environmental and psychological factors, including loss, isolation, personality characteristics and trauma, appear to play a strong role in MDD. While characterizing MDD as brain disorder may help destigmatize MDD, and environmental and social factors ultimately impact on the brain, the etiology of the disorder may not be 100% biological for some patients. Therefore, while there is a strong biological component and genetic vulnerability to MDD, I believe this disorder should not be seen solely as a neurobiological disorder, as it also draws attention from important societal issues and risk factors for MDD, such as chronic high work stress, or loneliness in the elderly.

### CONCLUSIONS:

- **Adult MDD is associated with structural and functional (network) dysconnectivity, possibly reflective of decreased white matter integrity and myelin damage.**
- **Studies suggest that MDD may be a neuroprogressive disorder, with damage increasing with every depressive episode, or brain abnormalities may represent a vulnerability marker for an unfavorable course of MDD.**
Methodological considerations

Direction of causation in cross-sectional analyses

Due to the cross-sectional nature of the analyses performed in this thesis, it is not possible
to discriminate between causes and consequences. In part I of this thesis, we speculated that
biological stress may result in altered brain morphology and thereby lead to vulnerability for
development of depression and/or anxiety disorders. However, it is also possible that altered
brain morphology precedes biological stress, by for instance decreasing feedback to the
HPA-axis. Of course, it is also possible that a third factor, which was not measured in our
study, for instance glucocorticoid level, is related to both biological stress markers and brain
morphology. In chapter 6 of this thesis, we only assessed functional and structural networks
at one timepoint, therefore, it remains unclear whether disease load of affective disorders
causes changes in brain networks or whether altered brain networks predispose individuals
to high disease load/an unfavorable course of MDD. Longitudinal studies are needed to
examine the direction of causality, while controlling for other related factors such as lifestyle
factors.

Inclusion of participants with and without psychopathology

In the first part of this thesis, we examined the association between biological stress and
brain morphology in a large sample, which includes both patients with a (history of) MDD
and/or anxiety disorders and healthy controls. We corrected for this in analyses, and overall
found similar associations in patients and controls in secondary analyses. However,
inclusion of both patients and controls may actually have been a strength of these studies,
as it guarantees a broad range of stress levels, which may make it easier to examine
associations.

Peripheral versus central measures of biological stress

It remains unclear whether measures of biological stress measured in peripheral blood
reflect these markers in the central nervous system. The blood-brain barrier separates
circulating blood from the brain and prevents entry of neurotoxins to the brain. Brain-
derived neurotrophic factor has been shown to cross the blood-brain barrier (Pan et al., 1998;
Pillai et al., 2010) and a correlation was observed between peripheral BDNF and BDNF in the
brain in young rats (Karege et al., 2002). Peripheral inflammatory markers can also enter and
affect the brain through multiple pathways. First, peripheral cytokines can be actively
transported through the blood-brain barrier. Second, inflammatory signals can be sent to
the central nervous systems through cellular signaling or signaling through the vagal nerve. The least evidence is available for an association between peripheral and central oxidative stress. Oxidants and anti-oxidants are difficult to measure in vivo due to their short half-life. In this thesis, we examined two more stable measures, 8-OHdG and F2-isoprostanes, which are measures of reactive oxygen species (ROS)-related damage to lipids and DNA. These measures thus represent the net effect of oxidative stress, circumvent the issues regarding short half-life, and are considered the best markers of oxidative stress at this moment (Halliwell, 2011). It remains unclear whether oxidative damage to peripheral cells correlates with central oxidative damage. To our knowledge, only one study has examined the association between these peripheral markers and central markers of oxidative stress. This study by Liu et al. (2004) found that in rodents changes in peripheral 8-OHdG in plasma reflected changes in oxidative stress in the brain following cerebral infarction. The findings suggest that peripheral 8-OHdG may be used as a biomarker of oxidative brain damage. More work is needed to confirm these findings.

Experimental studies in animals have been necessary to examine central inflammation and oxidative stress as these measures could not be assessed in humans in vivo. However, new neuroimaging methods are becoming available that will shed new light on neuroinflammation and central oxidative stress in humans in health and disease. For inflammation, new positron emission tomography (PET) markers are becoming available. TSPO, translocator protein 18 kDa is an indirect marker of neuroinflammation, and is highly expressed in macrophages, lymphocytes, activated microglia and astrocytes (Wu et al., 2013). First studies have revealed that TSPO is increased in the prefrontal cortex, anterior cingulate and insula of MDD patients (Setiawan et al., 2015; Holmes et al., 2018). Studies have also been done with radiolabeled cytokines including IL-2 and TNF-a, but these markers are still in the preliminary stages as imaging targets. While validation of these measures is needed, the advent of novel PET markers of neuroinflammation is a promising development in the field of inflammation.

Central oxidative stress may be assessed in vivo using magnetic resonance spectroscopy (MRS). MRS can be used to obtain biochemical information from a specific brain region. Many studies have examined glutathione (GSH) concentrations in the brain. Glutathione appears to be the most abundant antioxidant in living tissue (Cobb and Cole, 2015). Interestingly, the first studies show that MDD may be associated with increased glutathione in the ACC (Duffy et al., 2015), but decreased glutathione in the occipital cortex (Godlewska et al., 2015).

**Measuring biological stress markers at one timepoint**

The biological stress response is a dynamic and reactive process, which is influenced by
genetic, psychological, and environmental factors. Therefore the dynamic nature of these systems is not captured when we examine these markers at one time point. Few studies have multiple measurements of BDNF, inflammation or oxidative stress, therefore this is a limitation of all studies in the biomarker field. Brain structure is not only influenced by current biological stress, and may reflect the cumulative effect of biological stress markers. If there is large variability in these markers over time, this may increase noise in our biological stress measure. If the effect is strong and the sample size is large, an effect may still be observed, but small effects may be obscured. In the NESDA study, inflammation markers appear to be relatively stable over two years (data not shown). The stability of these markers is further highlighted by the strong genetic component and also the influence of relatively stable traits like body mass index. Less is known about the stability of oxidative stress markers over time and BDNF. The inability to capture the dynamic nature of biological stress mechanisms not only reflect a limitation of the studies in this thesis, but are a limitation in the study of biomarkers in psychiatry in general (Black et al., 2017).

Polygenic risk scores and neuroimaging

In chapter 4, we examined polygenic risk scores of CRP, triglyceride levels and BMI. Polygenic risk scores are calculated based on summary statistics from genome-wide association studies (GWAS), by summing the numbers of risk allele that a person has, weighted by the effect size from the GWAS. This is a measure of the genetic liability to a certain trait (Lewis & Vassos, 2017). Polygenic risk scores are dependent on the size of the discovery sample (the GWAS study). In chapter 4 polygenic risk scores for BMI were calculated on a GWAS study of 249,796 subjects, scores for CRP were calculated on a GWAS of 80,000 subjects and GWAS triglyceride data was available for 96,598 subjects. Since then, larger GWAS studies have been performed. A recent GWAS study of BMI contained data from 700,000 individuals (Yengo et al., 2018), while a more recent meta-analysis of triglyceride levels included 189,000 subjects (Willer et al., 2013). These larger samples have had more power to detect small effects and therefore the resulting polygenic risk scores will better represent the trait of interest. As the polygenic effects will likely have a small effect on a phenotype or endophenotype, a large sample size is needed to find associations between polygenic risk scores and traits or disorders. The study in chapter 4 may have been underpowered to detect small and robust associations. However, examining genetic factors in biomarker research in psychiatry remains a powerful approach for future studies, as the direction of causality is clear, as genes, in contrast to other factors, are stable across the lifetime.
Neuroimaging measures: region of interest (ROI) approach

In all studies described in this thesis, we used a region or tract of interest approach and did not perform analysis at the voxel-level. This approach has advantages and disadvantages. Disadvantages of an ROI approach are that averaging all voxels within a region may mask effects and limit spatial resolution. However, a ROI approach limits the number of tests, and thus reduces risk of type I errors), enhances signal-to-noise ratio by averaging out outliers, and provides robust measures that can be easily compared across different sites and studies.

In chapter 3 and 4, mean volume or thickness were examined across the left and right hemisphere as we expected similar associations between biological stress and brain morphology. This approach increased our power and further reduced the number of tests that were performed. In post-hoc tests, we examined whether significant effects were driven by the left or right hemisphere. Results of these analyses provide little evidence for a lateralized effect.

Neuroimaging measures: reliability of neuroimaging measures

The field of psychology and psychiatry, including neuroimaging in psychiatry, has been plagued by low reproducibility of findings (Poldrack et al., 2017). In neuroimaging, low reproducibility can be related to differences in acquisition methods and parameters, but even when the acquisition method is identical, factors related to scanning (e.g. changes in image orientation, magnetic field instability) or neuroimaging analysis methods and software may underlie low reproducibility of findings. Several studies have therefore examined whether certain neuroimaging measures are reliable and reproducible when the same subjects are scanned multiple times. This test-retest reliability of Freesurfer-derived subcortical volume (used in chapter 2-4) strongly varied across regions, with lowest reliability observed for the amygdala and highest reliability observed for the thalamus and caudate (Morey et al., 2010).

Reliability or reproducibility of fractional anisotropy values derived with the ENIGMA-DTI protocol used in chapter 5, was assessed in one study by scanning participants three times in one week and comparing FA measures from all scans. Overall, reliability or reproducibility was excellent across regions, which suggests that the ENIGMA protocol could be used to extract measures at multiple time points for longitudinal analysis of white matter changes (Acheson et al., 2017).

The test-retest reliability of graph measures derived from functional and structural networks (which was used in chapter 6) is less clear, as it depends on the tractography method, spatial resolution, sparsity range, and varies between graph measures (Dennis et al., 2012). To conclude, the reliability of neuroimaging measures depends on the modality, software
Clinical implications

Important goals in the field of biological psychiatry are to increase our knowledge of the pathophysiology of psychiatric disorders and to identify biological markers of these disorders. These biomarkers could potentially be used to aid differential diagnosis between disorders, identify those at risk, predict course or treatment response, aid in selection of treatment options, personalize treatment and further increase our understanding of the pathogenesis of the disorders. While some potential biomarkers of MDD have been identified, the mechanisms through which they may affect vulnerability to psychopathology remain unclear.

In the first part of this thesis, we examined how these potential biomarkers of depression were related to brain morphology. Our results reveal that decreased cortical thickness in the ACC may be one pathway through which immunometabolic dysregulation may impact on affective disorders. Rostral ACC morphology may represent an intermediate phenotype of immunometabolic dysregulation, showing stronger associations with MDD and increased stability compared to peripheral inflammation markers, however rostral ACC thickness is not specific or sensitive enough to MDD to be used for differential diagnosis in a clinical setting, but might perhaps be a marker for treatment response. It is well recognized that anti-inflammatory drugs may reduce depressive symptoms (Kohler et al., 2014), and we speculate that this treatment effect might be mediated by recovery of rostral ACC thickness. Although speculative, if this is the case, rostral ACC thickness might be clinically useful as a treatment biomarker. Replication and longitudinal studies are needed to examine how stress-related neuroimaging biomarkers are related to onset and course of MDD.

As mentioned earlier, neuroimaging findings in the second part of this thesis raise the question whether MDD is a neuroprogressive disorder. If this is the case, it further highlights the need for early treatment before more severe deficits can occur. However, these neuroimaging abnormalities may also precede the disorder and represent neuroimaging markers related to poor course of MDD, which then suggests that these markers could potentially be used to identify individuals at risk for a poor course of MDD, which may be related to poor treatment response. However, these findings are in need of replication and (neuroimaging) biomarkers of depression are far from the clinical use at this time.
Future studies

As mentioned throughout this discussion, longitudinal neuroimaging studies are urgently needed to disentangle causes and consequences of brain abnormalities in MDD. In addition, large-scale multi-site studies similar to the one discussed in chapter 5 in the ENIGMA consortium, will be needed to identify (neuroimaging) biomarkers of MDD that are robust and can be replicated across many different samples worldwide, thereby addressing the issue of poor replication in the field of biological psychiatry. As this sort of collaboration is becoming more common, new studies may want to harmonize data collection, including collection of biomarker, clinical and neuroimaging data, so that better harmonization in analysis across sites is possible. In addition to large-scale multi-site studies, smaller studies will also be needed for testing very specific hypotheses as these studies usually have more detailed phenotypic information compared to multi-site studies.

New neuroimaging methods and analysis techniques are also becoming available. As mentioned previously, an important antioxidant, glutathione, can now be measured in the brain in vivo using MRS and new PET markers of inflammation are also available. While measuring glutathione in the brain is hampered by technical difficulties and PET studies are expensive at this time, this may become more common in the future. Additionally, new analysis techniques are becoming available for analysis of white matter integrity, including free-water imaging (Oestreich et al., 2017; Pasternak et al., 2009). Fractional anisotropy measurements in traditional DTI analyses, such as presented in chapter 5, may potentially be biased by extracellular free-water, water molecules that are not restricted by tissue and diffuse freely in the extracellular space, which is most often seen in cerebrospinal fluid. Differences between tissues and patients in free-water may affect anisotropy measures and free-water imaging corrects for this and results in free-water corrected fractional anisotropy measures (Pasternak et al., 2009). Free-water correction may increase sensitivity to finding differences in white matter integrity between MDD patients and controls (Bergamino et al., 2016).

Finally, one topic that was not addressed in this thesis, and is important for future studies in the field, is the heterogeneity of MDD and anxiety disorders. There is much variability across MDD and anxiety patients in symptoms, course and risk factors. Even the underlying pathophysiology may differ between patients. This variation may limit our ability to detect associations or effects in a large study sample, while these effects or associations would be seen in subgroups. Subtyping of patients is therefore important in moving forward, and can be done based on symptom clusters (Lamers et al., 2010) or biomarkers (Drysdale et al., 2017). While creating distinct subgroups of patients based on symptoms or biomarkers may not be feasible, an alternative approach in line with the Rdoc approach, could be to use this information to create subdimensions of MDD. Identification of valid subtypes or
Subdimensions of MDD and anxiety disorders on the basis of biomarkers, for instance identifying MDD patients with high inflammation, may guide treatment options and advance the field towards personalized medicine and better treatment outcome.

Conclusions

Affective disorders are neuropsychiatric disorders, with an extremely complex and multifactorial etiology and pathophysiology. The work presented in this thesis adds to our understanding of these disorders by revealing new associations between stress-related biomarkers, affective disorders and neuroimaging measures of brain structure. The findings suggest that deficits in brain structure may be one pathway through which biological stress impacts affective disorders, revealing a potential intermediate (neuro)phenotype of inflammation. The findings also show that structural and functional dysconnectivity of brain networks play an important role in the pathophysiology of these disorders, but large-scale longitudinal studies are still warranted to examine if these abnormalities precede the onset of affective disorders. Furthermore, our finding also point to the relevance of taking environmental factors (e.g. lifestyle, trauma) into account when studying the biological basis of affective disorders, as environmental factors and genetic factors interact to increase vulnerability to these disorders. Moving forward, the field of biological psychiatry would not only benefit from deeper, detailed clinical and biological phenotyping, but also examining subdimensions of biological and relevant environmental factors. Increasing our understanding of the contribution of biological and environmental factors and underlying mechanisms, will move the field further to the ultimate goal in psychiatry, offering more effective treatment options to improve the quality of life for those suffering from mental illness.
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