1 General introduction
General introduction

Major depressive disorder

Symptoms and prevalence

Major depressive disorder (MDD) is a psychiatric disorder characterized by loss of interest and enjoyment of everyday experiences, low mood and emotional, cognitive and neurovegetative symptoms. It is a common disorder, with lifetime prevalence estimated between 14 and 20% (Bromet et al., 2011; WHO, 2003). MDD negatively impacts the daily functioning and quality of life of patients and is predicted to be the leading cause of disability in high-income countries by 2030 (Matthers et al., 2006). Recurrence of depressive episodes is common, with 80% of patients experiencing more than one episode (Vos et al., 2004), which further adds to the high disease burden.

Comorbid anxiety disorders

There is a strong comorbidity between MDD and anxiety disorders (including generalized anxiety disorder, panic disorder and social phobia), with estimates varying between 30-50% (Kessler et al., 1996; Beekman et al., 2000). Patients with depression and comorbid anxiety disorders show increased functional impairment, severity of symptoms and chronicity of depression and anxiety compared to patients with only MDD or an anxiety disorder (Penninx et al., 2015).

Treatment of MDD and anxiety disorders

Treatment of MDD and anxiety disorders currently consists of psychotherapy and/or pharmacological treatment. Cognitive behavioral therapy (CBT) is a widely used psychotherapeutic intervention used to treat both MDD and anxiety disorders, although its effectiveness in children and adolescents remains unclear (Cox et al., 2014). Pharmacological treatment of MDD and anxiety disorders includes use of selective serotonin re-uptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). First generation antidepressants including tricyclic antidepressants (TCAs) and monoamine-oxidase inhibitors (MAOIs) are also still used to treat MDD patients who do not respond to use of SSRIs or SNRIs. Psychotherapy and pharmacological treatments appear to be equally effective in the treatment of MDD and anxiety disorders (Eddy et al., 2004; Vos et al., 2004), but a combination of both may have the largest effect (Pampaliona et al., 2004). Not all patients respond to treatment, as an estimated 30% of patients do not reach remission of MDD after several treatment attempts with medication and optional CBT (Rush et al., 2006). Therefore,
it remains important to further elucidate the pathophysiology and etiology of these disorders to identify new treatment targets.

**Etiology and risk factors**

MDD is a complex disorder in which genetic, epigenetic and environmental factors, such as stress, together contribute to development of the disorder (Dean & Keshavan, 2017). Heritability of MDD is estimated around 35% (Sullivan et al., 2000). In most cases, a genetic predisposition towards MDD is not sufficient to cause MDD, and environmental stress factors also appear to play an important role. These environmental stress factors include negative life events (such as divorce or death of a relative), daily hassles and unfavorable psychosocial circumstances including poverty or widowhood. Stress has been associated with the onset (Kendler et al., 1999; Daley et al., 2000), severity (Hammen et al., 1992) and course of MDD (Kendler et al. 1997). Furthermore, early life stress, including childhood abuse and neglect, also increases the risk of developing MDD and anxiety disorders in later life (Scott et al., 2012; Li et al., 2016) and is associated with more severe symptoms, poorer course and treatment resistance (Hovens et al., 2012). Although considered distinct disorders, anxiety disorders closely resemble depression in terms of genetics, shared environmental risk factors and pathophysiology (Spinhoven et al., 2010; Penninx et al., 2015; Lee et al., 2013).

**Part I: The involvement of biological stress systems in psychiatric conditions**

**Biological stress systems**

As described above, environmental stress factors constitute important risk factors for development of MDD and anxiety disorders. The word ‘stress’ is commonly used to describe feelings of strain or pressure in response to crises, major life events or everyday hassles. We experience stress in situations when our coping strategies appear to be inadequate to adapt to a threatening situation. The human body reacts to stress by switching from a relaxed state to a ‘fight-or-flight’ state, by releasing various hormones and neurotransmitters, which then allows an individual to react to the threatening situation by either fighting or fleeing.

In response to stress the hypothalamic-pituitary-adrenal (HPA) axis is activated, resulting in cortisol, or glucocorticoid, release from the adrenal gland. Cortisol increases cardio-vascular activity, metabolism and production of glucose, needed for energy. Cortisol also regulates the stress response, as it provides feedback to the HPA-axis, which can in turn terminate the stress response. The sympathetic nervous system is also active in response to stress, leading to an increase in epinephrine and norepinephrine levels and a decrease in acetylcholine, which in turn leads to an increase in the release of pro-inflammatory...
cytokines, including interleukin-1 (IL-1) and interleukin-6 (IL-6) (Steptoe et al., 2007; Weik et al., 2008). Longitudinal work shows that psychological stress predicts an accelerated increase in IL-6 levels (Kiecolt-Glaser et al., 2003). There is also evidence that stress-induced activation of the HPA-axis disrupts the cellular balance of reactive oxidant species (oxidants) and anti-oxidants, thereby causing an increase in oxidative stress (Schiavone et al., 2013). Thus, stress, amongst others, causes HPA-axis activation, an increased release of pro-inflammatory cytokines and increased oxidative stress. These are not independent effects, as all mechanisms bidirectionally interact.

**Biological stress and major depressive disorder**

The physiological or biological stress response is usually beneficial, as it helps us adapt to new environments and maintain homeostasis. However, chronic activation of these biological stress systems has long-term and negative implications for health. Dysregulation of the biological stress systems, including hyperactivity of the HPA-axis, increased neuroinflammation, oxidative stress and decreased BDNF, has also been implicated in the pathophysiology and etiology of MDD (see Dean & Keshavan, 2017 for a review). For instance, depressed individuals show an abnormal HPA-axis response to stress, including hypersecretion of cortisol releasing hormone (CRH), increased cortisol levels in urine, saliva and blood plasma, impaired negative feedback to the HPA-axis, enlarged pituitary and adrenal glands (Pruessner et al., 2003; Stetler and Miller, 2011; Nemeroff & Vale, 2005). High cortisol levels may also be a risk factor for onset of MDD (Goodyer et al., 2000).

Pro-inflammatory cytokines, including IL-6 and IL-1, and markers of inflammation like C-reactive protein (CRP) were also increased in peripheral blood of MDD patients (Howren et al., 2009; Dowlati et al., 2010). Increased inflammation was found to be a risk factor for the onset of MDD (Benros et al., 2013; Valkanova et al., 2013) and increased levels of IL-6 in childhood were found to increase the risk of MDD in young adulthood (Khandaker et al., 2014). Decreased anti-oxidant levels and increased oxidative damage in MDD (Liu et al., 2015; Black et al., 2015) and decreased peripheral BDNF levels (Molendijk et al., 2014) in MDD patients have also been reported.

**Biological stress and the brain**

Dysregulation of the biological stress systems (e.g. high cortisol levels, increased pro-inflammatory cytokines and increased oxidative stress) may be associated with the onset, severity and course of MDD by their impact on the brain.

The brain is protected from peripheral neurotoxins by the blood-brain barrier (BBB), which separates circulating peripheral blood from the central nervous system. Peripheral
pro-inflammatory cytokines however, can cross the BBB in a number of ways, including through active transport and inflammation-related decreased permeability of the BBB. An increase in pro-inflammatory cytokines in the brain causes an increase in neurotoxic kynurenine pathway metabolites, including quinolinic acid, and causes damage to neurons (see review by Kim and Won, 2017). These neurotoxic metabolites also promote oxidative stress, neuronal apoptosis and glutamate excitotoxicity (Perez-de la Cruz et al., 2012), which also damages neurons and glia cells. High levels of cortisol and chronic exposure to stress have been associated with loss of synapses in neurons and altered function of glia cells (Margarinos & McEwen, 1995; Jauregui-Huerta et al., 2010).

Chronic biological stress may not only cause neurodegeneration, but may also lead to decreased neurogenesis. New nerve cells are formed in the hippocampus from pluripotent stem cells even after birth (Fuchs & Gould, 2000). One peptide that plays an essential role in neurogenesis, neuronal survival and plasticity is Brain-derived neurotrophic factor (BDNF), which is expressed in the hippocampus and cortex (Park & Poo, 2013; Numakawa et al., 2017). Chronic stress may negatively impact BDNF levels, as administration of cortisol has been shown to decrease BDNF expression in hippocampal and cortical regions (Smith et al., 1995; Gourley et al., 2009). Early life stress, which includes abuse or neglect, has also been associated with decreased expression of BDNF in animal studies (Bondar & Merkulova et al., 2016) and may lead to a stress-related decrease in neurogenesis and neuroplasticity. Decreased hippocampal neurogenesis may further increase biological stress by causing a decrease in cortisol-mediated negative feedback to the HPA-axis, leading to prolonged HPA-axis activity and further changes in brain morphology.

Chronic stress and subsequent dysregulation of the biological stress systems may contribute to the onset, severity and course of MDD by causing an increase in neurodegeneration and decrease in neurogenesis in brain regions involved in emotion regulation. Various brain regions that play a role in emotion regulation and have been associated with depression, including the hippocampus, amygdala and anterior cingulate cortex (ACC), may be especially vulnerable to inflammation and oxidative damage.

The hippocampus for instance is a limbic brain structure that is crucial for learning and memory and is functionally and structurally connected to the amygdala and prefrontal cortex. This region is vulnerable to the effects of biological stress, as it contains many glucocorticoid receptors (Sapolsky et al., 1984). In animal studies, chronic stress has been shown to decrease dendritic branching, neurogenesis and plasticity in the hippocampus (Son et al., 2012; Masi & Brovedani, 2011). In rodents, decreased hippocampal neurogenesis was associated with depressive symptoms and anxiety-related behavior (Murray et al., 2008). Interestingly, rodent studies suggest that chronic stress may enhance plasticity and dendritic branching in parts of the amygdala, a limbic brain region involved in emotion processing and fear conditioning (Kuhn et al., 2014; Marsden, 2013). Furthermore, glucocorticoid
administration in neonates increased plasticity in the amygdala and led to depressive behavior in adult rats (Ko et al., 1995). Finally, the anterior cingulate cortex, a prefrontal area involved in both cognition and emotion regulation, may also be vulnerable to effects of biological stress. This region has many glucocorticoid receptors (Radley et al., 2004) and animal studies have shown loss of neuronal dendrites and spines in response to chronic stress in this region (Radley et al., 2008).

**Associations between biological stress markers and brain in human neuroimaging**

Evidence for an association between these biological stress markers and brain structure in humans is lacking or is based on small samples. While it is not usually possible to examine morphology at the neuronal level *in vivo* in humans, neuroimaging techniques allow us to examine regional brain volume.

Previous studies have examined the relationship between BDNF and regional brain morphology. As BDNF is associated with both neurogenesis and with stress, decreased BDNF in individuals with (a history of) severe stress, and possible subsequent changes in brain structure, may underlie the development of stress-related psychopathology. Neuroimaging studies have focused on the BDNF gene and especially the Met-allele of the Val66Met single nucleotide polymorphism (SNP). Individuals with the met-variant of this gene show decreased secretion of BDNF (Egan et al., 2003; Chen et al., 2004). Neuroimaging studies report an interaction effect between early life stress or childhood maltreatment and the met-variant of the BDNF gene with brain structure of the amygdala, hippocampus and prefrontal cortex (Gerritsen et al., 2012; Molendijk et al., 2012; Gatt et al., 2009), suggesting that individuals with the Met-genotype (and thus possibly lower BDNF levels) are more vulnerable to the effects of early stress on the brain. However, it remains unclear how BDNF levels in peripheral blood are associated with brain morphology in individuals with a history of maltreatment. Therefore, this thesis examines how the BDNF gene, BDNF gene expression and BDNF protein levels are related to childhood maltreatment and interact with maltreatment to affect brain morphology.

Several neuroimaging studies have examined the association between inflammation and brain function and structure. High levels of peripheral IL-6 and CRP were associated with lower hippocampal volume (Satizabal et al., 2012). Furthermore, stress exposure was associated with an increase of IL-6 and TNF-α levels and an increase in these markers was associated with greater activity of the anterior cingulate, insula and amygdala (Slavich et al., 2010; Muscatell et al., 2015). Inflammation has also been associated with metabolic dysregulation, for instance abdominal obesity, low HDL-cholesterol and hypertriglyceridemia. While metabolic dysregulation has been associated with changes in brain morphology (Alosco et al., 2014), it remains unclear whether both inflammation and
metabolic dysregulation affect similar brain regions. Therefore in this thesis, the association between markers of inflammation and metabolic dysregulation – together referred to as immunometabolic dysregulation – and brain morphology is examined, while additionally correcting for relevant lifestyle factors.

To our knowledge, only one neuroimaging study has examined the association between oxidative stress and brain morphology in humans. Lindqvist et al. (2014) calculated a ‘total net antioxidant score’, which consisted of oxidant and anti-oxidant markers, and was positively associated with hippocampal volume. This study examined a small sample of 35 participants and did not correct for lifestyle or medication use factors. Examining associations between oxidant markers and brain morphology is difficult, as the half-time of oxidants is short. One way to circumvent this issue, would be to look at the cellular effects of long-term exposure to oxidative stress on brain morphology. 8-hydroxy-2’-deoxyguanosine (8-OHdG) and 8-iso-prostaglandin F2α (F2-isoprostanes) are measures of oxidative DNA and lipid damage. No previous studies have examined the association between these measures and brain morphology in humans, therefore this thesis examined whether these measures are related to hippocampal and amygdala volume and whether we can replicate the findings by Lindqvist et al. by using these new markers of oxidative stress.

Part II: Neuroimaging of depression

Neurocircuitry of depression

The advent of neuroimaging to psychiatry has brought the ability to investigate the pathophysiology of depression in vivo, by examining brain function, structure and connectivity of different brain regions in patients. Identifying reliable neural biomarkers of depression is important, as it not only increases our understanding of the mechanisms underlying MDD, but may also lead to new treatment targets and, in the future, may help discover neuroimaging biomarkers that predict course of depression.

An early model implicates the (para)-limbic circuits in mood regulation deficits and the etiology of depression and anxiety disorders (Mayberg et al., 2003). According to this model, limbic regions including the amygdala, hippocampus, and basal ganglia process emotions, while prefrontal regions (i.e. subgenual, medial and dorsolateral prefrontal cortices) regulate the limbic system. Depression and anxiety disorders may result from increased limbic activation or deficits in prefrontal top-down control over these limbic regions (Mayberg et al., 2003; Dean & Keshavan, 2017). An alternative model suggests increased or sustained activation in a network of regions including the medial prefrontal cortex, precuneus, lateral temporal cortex and lateral parietal cortex may play a role in rumination and MDD symptoms (Drevets et al., 2008, Greicus et al., 2007).
Structural and functional brain alterations in MDD

It is possible to examine these brain regions in depressed patients using structural and functional MRI (fMRI). In structural MRI studies, volume, thickness and surface area of cortical and subcortical brain regions can be examined and compared between patients and healthy controls. Functional MRI is used to examine brain activity, by detection of changes in regional blood flow. This is done by looking at the blood-oxygen-level dependent (BOLD) response and is based on the premise that neurons in active brain regions use more oxygen, causing an increase in oxygen saturation in the blood and causing the MRI signal to increase. Functional MRI has been used to examine brain activity related to performance of specific cognitive or emotional tasks (task-based fMRI) but can also be used to examine brain activity during rest (resting-state fMRI or rsfMRI). Brain activation during performance of a task or during rest can then be compared between patients and controls.

In line with models of the neurocircuitry of MDD, structural MRI studies have shown decreased volume in prefrontal brain regions, including the ACC, dorsomedial, dorsolateral and orbitofrontal cortex, and subcortical regions including the hippocampus, amygdala and striatum (Koolschijn et al., 2009; Videbech & Ravnkilde, 2015; Bora et al., 2012). Findings from structural imaging studies in depression have often been inconsistent, partly due to the heterogeneity of depressive disorders, differences in acquisition methods and limited power due to small sample sizes. Meta-analyses from the MDD working group of the Enhancing Neuro-Imaging Genetics though Meta-Analysis (ENIGMA) consortium, including data from more than 2000 MDD patients and 8000 healthy controls, have shown a subtle decrease in volume of the hippocampus in patients with recurrent MDD (Schmaal et al. 2016) and widespread cortical thinning in the orbitofrontal cortex, anterior and posterior cingulate, insula and temporal lobes (Schmaal et al., 2017).

Functional studies of MDD have shown decreased prefrontal activity, predominantly in the medial prefrontal cortex, and increased or sustained activity in limbic regions including the amygdala during emotional tasks (Siegle et al., 2002; Sheline et al., 2001; Hamilton et al., 2012; Groenewold et al., 2013). During cognitive tasks, patients may show increased compensatory prefrontal and ACC activation (Harvey et al., 2005; Fitzgerald et al., 2008). Findings have been inconsistent across studies and a meta-analysis examining which regions consistently show abnormal brain activation in MDD during cognitive tasks and during emotional tasks failed to find brain abnormalities that overlapped between studies, possibly due to differences in methodology or disease heterogeneity (Muller et al., 2017).

Abnormal functional and structural brain networks

It is now recognized that complex behavior, including cognition and emotion-regulation, is
not dependent on activation of a single brain region, but is achieved through recruitment of multiple brain areas that are functionally and structurally connected. Recent technological advances in neuroimaging have now allowed us to examine the brain as a network of connected brain regions. The field of psychiatric neuroimaging has therefore advanced from small studies focused on regional brain structure and function in depression to more complex, network-based approaches.

Cortical and subcortical brain regions are structurally connected through white matter (WM) tracts. Abnormalities in these WM tracts may cause a disconnection between brain regions and underlie MDD symptoms. These structural brain networks can be studied using diffusion tensor imaging (DTI), which characterizes the directionality of water diffusion in the brain and thereby allows us to examine WM microstructural properties in vivo. A common measure used in DTI research is fractional anisotropy (FA). FA ranges between 0 and 1, where a high value represents more directionally constrained diffusion of WM, likely due to intact myelination and compactness of fiber bundles. Lower FA represents less constrained diffusion, possibly due to WM damage. Studies in patients with MDD have shown decreased WM integrity (i.e. lower FA) in the corpus callosum, the WM tract that connects both hemispheres of the brain (Liao et al., 2013; Murphy & Frodl, 2011). Lower FA has also been reported in other regions including the internal capsule and uncinate fasciculus (LeWinn et al., 2014; Zou et al., 2008; Choi et al., 2014). It remains difficult to interpret these inconsistent findings, as there are large differences between studies in methodological approaches and sample sizes. Furthermore, it remains unclear whether these findings are driven by specific groups of patients and whether the findings are associated with clinical characteristics of depression. Therefore, in this thesis, the largest meta-analysis to date is performed, including data from 20 sites worldwide, which have used DTI to examine differences in WM tracts in MDD patients. This meta-analysis is not based on the available literature, but is based on results from 20 sites worldwide (with a total of 1300 patients and 1600 controls), which use harmonized protocols for processing of DTI data and statistical analysis. Besides looking at differences in WM between patients and controls, we also examine how WM deficits are related to clinical characteristics.

A meta-analysis of studies that have examined functional brain networks during rest has found decreased connectivity within three important brain networks: the frontoparietal network, the default mode network and the salience network. The frontoparietal network is involved in cognitive control and emotion regulation, whereas the salience network is involved in bottom-up direction of attention to salient stimuli (Kaiser et al., 2015). Connectivity within the default mode network (DMN) appears to be increased in MDD, and may be related to rumination or increased internal thoughts (Kaiser et al., 2015; Greicus et al., 2007).
While these abnormalities have been described in both functional and structural networks in MDD patients, it remains unclear if these deficits are related to disease load of MDD. While previous work has found associations between deficits in these networks and measures of disease load, including disease duration and number of episodes, these measures do not fully capture the recurrent and remitting nature of MDD or anxiety disorders. In one chapter of this thesis, we therefore examine functional and structural networks using DTI and rsfMRI and determine the percentage of time with MDD and/or anxiety symptoms over nine years prior to scanning. This percentage is then used as a measure of disease load and is associated with network characteristics of functional and structural networks.

**Aims and outline of this thesis**

In the first part of this thesis, the association between peripheral markers of biological or physiological stress (inflammation and oxidative stress) in relation to regional brain structure is examined. Furthermore, we examine the relationship between BDNF (measured at the gene, RNA and protein level) in interaction with childhood trauma and brain morphology. As we are interested in ‘general’ associations, these analyses are performed across patients and controls, providing us with a large variation in these biological markers. Secondary analyses examine whether these associations are different in patients and controls using interaction analyses.

In the second part of this thesis, differences in functional and structural brain networks between patients and controls are examined. First, we present a prospective meta-analysis of diffusion tensor imaging studies performed within the MDD working group of the ENIGMA consortium, in which differences in white matter integrity between 1305 patients and 1602 healthy controls are examined. We also examine how these WM structures are related to clinical characteristics of MDD. Second, the effect of disease load of MDD and anxiety disorders on characteristics of structural (DTI-based) and functional (resting-state functional MRI based) brain networks are examined using graph theoretical analysis.

The main objectives of the thesis are:

1. To examine the association between BDNF (at the gene, gene expression and protein level) and brain morphology and to examine if this association is different in individuals with a history of childhood maltreatment (chapter 2).
2. To examine the association between biological stress markers and regional brain morphology (immunometabolic stress chapter 3 & oxidative stress chapter 4).
3. To examine alterations in structural and functional brain networks associated with depression and in relation to clinical characteristics of the disease (meta-analysis chapter 5; brain networks and load of affective disorders chapter 6).
Cohorts studied in this thesis

Netherlands Study of Depression and Anxiety

The Netherlands Study of Depression and Anxiety (NESDA) is a longitudinal study that examines the course and consequences of depression and anxiety. At the baseline measurement, which took place between 2004 and 2007, 2981 participants were included, of which 78% met criteria for MDD and/or an anxiety disorder. All participants were recruited from the community, primary care and from specialized mental health care. Follow-up assessments were performed two, four, six and nine years after the baseline measurement. The neuroimaging study within NESDA includes data from 302 participants at the baseline measurement, and 140 subjects at the nine-year measurement.

Strengths of the NESDA study include the large sample size and longitudinal nature of the study. Furthermore, there is a detailed assessment of demographic, clinical, genetic, psychosocial and biological measures, allowing us to correct for possible confounders in our analyses.

Enhancing Neuroimaging Genetics through Meta-Analysis consortium

The Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) consortium is a worldwide collaboration with the aim of increasing our understanding of brain function, structure and disease by examining and combining genetic and neuroimaging data. Currently the Major Depressive Disorder Working Group within the ENIGMA consortium includes data from more than 30 research sites worldwide, with neuroimaging data available for 3244 patients and 4909 controls. The main aim of the working group is to use meta- and mega-analytic techniques to identify structural and functional neuroimaging markers that differentiate MDD patients and healthy controls. All research sites involved in ENIGMA projects use standardized and harmonized protocols for processing of neuroimaging data, quality assurance and statistical analyses, which then allows direct comparisons across studies and meta-analysis.
References


