Chapter 1

General introduction
DEPRESSIVE AND ANXIETY DISORDERS

Epidemiology
Depressive and anxiety disorders (generalized anxiety disorder (GAD), panic disorder, agoraphobia, social phobia, specific phobia and obsessive compulsive disorder) are common mental disorders with lifetime prevalences of 19.0 and 19.3%, respectively, in the Netherlands\(^1\). The World Health Organization predicts that by the year 2020, depression will rank second of the most disabling diseases in the world, after cardiovascular disease\(^2\). These disorders cause a great burden on the patient, people in their environment specifically and society in general, affecting social and work functioning\(^3,4\) and resulting in high costs\(^5\). This burden is in part due to their course, since recurrence and chronicity are common for depression and anxiety disorders. Approximately 20% of depressive disorders have a chronic course and most patients experience recurrent episodes\(^6,7\). For anxiety disorders, the course appears to be even less favorable\(^8,9\). The anxiety disorders with the highest prevalence in combination with a high burden of illness are: GAD, panic disorder with or without agoraphobia, and social phobia, which are examined in this thesis. Also, a higher prevalence of cardiovascular disease and diabetes have been observed for depressive and anxiety disorders\(^10\), which further adds to the burden of illness and for which the underlying mechanisms are not completely elucidated yet. In addition, comorbidity between depression and anxiety disorders is high (30-60%)\(^11-13\); they share symptoms and potentially also pathophysiological processes. Therefore, these diseases need to be studied in tandem.

Clinical symptoms
This thesis focuses on major depressive disorder, GAD, panic disorder with and without agoraphobia and social phobia. In essence, major depressive disorder is characterized by a depressed mood and/or loss of interest or the ability to experience pleasure (anhedonia) for at least a two week period. Additional symptoms can include: weight gain or weight loss, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness and/or guilt, problems with concentration, indecisiveness, thoughts of death and suicidal ideation.

Anxiety disorders include disorders with situational (social phobia, panic disorder) and/or generalized anxiety (GAD) and are mostly characterized by symptoms of arousal and/or avoidance. Besides these general features, specific symptomatology exists. The main feature of GAD is "excessive anxiety and worry" about a variety of events and situations. Panic disorder is characterized by the occurrence of panic attacks and is in approximately 35-65% of the cases accompanied by agoraphobia\(^14\). Agoraphobia is an intense fear of being in certain situations in which escape is difficult or potentially embarrassing, or where help is
not readily available. The essential feature of social phobia is a marked and persistent fear of social or performance situations in which embarrassment may occur.

**Pathophysiology**

Depression and anxiety disorders are very heterogeneous and multicausal, a consequence of a complex interplay between genetic and environmental factors of which the pathophysiology is not completely understood yet. Heritability of major depressive and anxiety disorders accounts for around 30-40% of all cases\textsuperscript{15,16}. Apart from psychosocial factors (e.g. early life stress, life events, personality features) that may play a role in the etiology and course of depression and anxiety disorders, there has been growing attention towards biological correlates of these disorders over the past decades. The main hypothesis is the monoamine-deficiency-hypothesis, which states that levels of serotonin and/or norepinephrine are deficient in depressive and (possibly also) anxiety disorders. Next to the monoamine-deficiency-hypothesis, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in depression has received the most attention. One of the main reasons for this attention is that stress is associated with the onset and course of depression (and anxiety disorders) and drives the HPA axis.

**STRESS**

The concept of stress has evolved since the late 19\textsuperscript{th} century. Claude Bernard, Walter Cannon and Hans Selye provided key concepts for the current view\textsuperscript{17}. Hans Selye described stress as “a non-specific response of the body to any demand placed on it”\textsuperscript{18}. The term ‘stress’ is used in several definitions and is often used for both the stress response of the body and the stressor causing it. The stress response is considered a response to threatened homeostasis, following exposure to extrinsic or intrinsic adverse forces (stressors)\textsuperscript{19}. Stress (response) systems, such as the HPA axis enable a person to cope with the demands of a stressful event\textsuperscript{20}. A stressor is regarded harmful when there is a discrepancy between the demands and the ability to cope with it. Furthermore, the adaptive stress response may turn maladaptive with potential harmful consequences, when chronically stimulated\textsuperscript{20} (see also the HPA axis).

Stressors are thought to play a role in the onset and course of depressive disorder and anxiety disorders, since studies have reported a causal relationship between stressful life events, chronic daily hassles and childhood trauma and the onset and course of these disorders\textsuperscript{21-24}. However, since stressful events do not automatically result in psychopathology, it is hypothesized that a person’s vulnerability to stress is essential in the development of psychopathology\textsuperscript{25}. 
THE HYPOTHALAMIC-PITUITARY-ADRENAL (HPA) AXIS

The HPA axis is the main neuroendocrine system that is activated in response to stress, and consists of elements of the hypothalamus, pituitary and adrenal cortex (see Figure 1). In response to stress, via the cerebral cortex and amygdala, neurons in the paraventricular nucleus (PVN) of the hypothalamus release corticotrophin-releasing hormone (CRH) (and vasopressin). After release to the hypophyseal portal system, CRH stimulates the production of adrenocorticotropic hormone (ACTH), which is, in turn, released into the bloodstream. ACTH promotes the release of cortisol from the adrenal cortex into the systemic circulation. This system has many feedback loops to maintain homeostasis. The main feedback occurs at the level of the glucocorticoid (GR) and mineralocorticoid (MR) receptor in the pituitary and hippocampus. Cortisol enables a person to react to stress by mobilization of stored energy, suppression of immune function and facilitation of many processes of the central nervous system, such as memory and learning. These short-term effects of cortisol are necessary in order to respond to stress. However, chronically increased cortisol or CRH levels could also have deleterious effects, such as anxiety, changes in appetite and sleep, cognitive disturbances and lowered mood, decreased sensitivity to insulin, hyperlipidemia, hypercholesterolemia and immune destabilization. In addition, it has been reported that long-term HPA axis dysregulation may increase the risk of cardiovascular disease, stroke and diabetes.
Under normal conditions cortisol levels follow a diurnal rhythm, with high levels in the early morning, which gradually decrease during the day. Then, cortisol levels gradually increase again during the night. Superposed on this rise in cortisol levels is an extra peak upon awakening, called the cortisol awakening response (CAR). The CAR is a distinct response to awakening with peak levels normally around 30 minutes after awakening\textsuperscript{32,33}. It has been hypothesized that the CAR is associated with the anticipation of upcoming demands of the day\textsuperscript{34}. It is shown to be unrelated to the diurnal rhythm of cortisol, and is under genetic control unlike the rest of the diurnal cortisol levels\textsuperscript{35,36}, possibly due to polymorphisms in GR and MR receptors\textsuperscript{37}. Therefore, the CAR represents a distinct measure of HPA axis activity. Cortisol levels are generally low at the end of the day before night time, indicative of a basal activity level of the HPA axis. To examine the function of the negative feedback loop, the dexamethasone suppression test (DST) is used. Dexamethasone is a synthetic glucocorticoid that decreases cortisol levels by acting mainly on the pituitary\textsuperscript{38}, by binding to the GR, which has a higher affinity for dexamethasone than for endogenous glucocorticoids\textsuperscript{26}. When the feedback is adequate, cortisol levels are suppressed after dexamethasone intake. Lately, low doses of dexamethasone – 0.25 mg or 0.5 mg – tend to be used since these doses can discriminate between subtle disturbances of the negative feedback system of the HPA axis.

Cortisol is a low weight lipophile molecule. Therefore it can diffuse passively and can be measured in all bodily fluids, e.g. blood, liquor, urine and saliva. In blood, it is mainly bound to cortisol binding globulin and albumin. Unbound free cortisol is the biologically active form. Cortisol has increasingly been measured in saliva, since this returns the active unbound form of cortisol and can be obtained during a stress-free method under normal conditions, as respondents can provide saliva samples themselves and obtain them at home. In addition, saliva sampling allows for multiple samples during the day, including the CAR, thereby capturing the dynamics of the HPA axis. If the CAR is affected, this is an indication that HPA axis activity is also affected. On the other hand, when the CAR is not different between groups, it might still be that HPA axis activity is altered, since the sensitivity of the adrenal gland might be altered, resulting in higher levels of CRH and ACTH to maintain similar cortisol levels. Therefore, by measuring the CAR, it is not possible to measure sensitivity of the adrenal gland.

Cortisol levels are influenced by many internal as well as external stimuli, such as sex, age, socioeconomic status, smoking, physical activity, use of medication and many other factors\textsuperscript{39}. This may in part explain the inconsistencies found in previous smaller scale studies. The present study is designed to take these factors into account.
The HPA axis in depressive and anxiety disorders

Dysregulation of the HPA axis is thought to play a role in the pathophysiological basis of depression and – to a lesser extent – anxiety disorders\textsuperscript{27,40,41}. Several observations support this hypothesis. Cushing’s syndrome, which is characterized by extreme hypercortisolism, often presents itself with depression. Symptoms of HPA axis dysregulation, such as changes in appetite and sleep, loss of libido, are similar to some symptoms of depression. CRH injected intra cerebrally into rodents, can induce anxiety and depression-like symptoms\textsuperscript{42,43}. Corticosteroid medication can induce irritability and depressed mood, whereas antidepressants have shown to normalize the HPA axis dysregulation\textsuperscript{44}. Dysregulation of the HPA axis could also (partly) explain the link between depression and anxiety disorders on the one hand, and cardiovascular diseases on the other.

Indeed, there is a large body of literature linking HPA axis dysregulation to depressive disorders. Hyperactivity of the HPA axis in depression has even reached textbook status. Although not always consistent, hyperactivity of the HPA axis in depression has been reported since the 1960s, especially in severely depressed inpatients and was mostly reflected by increased CRH, ACTH in blood, cortisol levels in blood or urine and a decrease of the negative feedback using the DST\textsuperscript{38,45-48}. More recently, the HPA axis is assessed using the Dex/CRH test and salivary samples. Studies examining the Dex/CRH test and depression, report higher cortisol responses in depressed patients, especially in severely ill inpatients\textsuperscript{49,50}, but this has not consistently been observed in outpatients\textsuperscript{49-54}. Although higher salivary cortisol levels, such as the CAR, have been associated with depression\textsuperscript{55,56}, there are quite some conflicting results as well\textsuperscript{57,58}. Inconsistencies in results could possibly be explained by differences between studies, such as adjustment for confounding variables, type and severity of depression, use of psycho-active medication, and comorbidity with anxiety disorders. Higher cortisol levels have most frequently been reported for inpatients with severe melancholic or psychotic depressions\textsuperscript{59,60}, but studies in depressed outpatients show little difference in cortisol levels compared to persons without depressive disorders\textsuperscript{59,61,62}. Therefore, despite the large amount of studies, many uncertainties concerning the role of the HPA axis in depression remain.

The role of the HPA axis in anxiety disorders has been less extensively studied, except for PTSD, where no association or lower cortisol levels have been observed\textsuperscript{63}. For panic disorder results are inconsistent and the relationship between GAD or social phobia and the HPA axis has not been sufficiently addressed. For panic disorder, some studies reported higher basal cortisol levels\textsuperscript{64-67}, while other reported no difference\textsuperscript{68,69}. In addition, studies showed normal suppression\textsuperscript{70,71} or more non-suppression after dexamethasone ingestion in panic disorder\textsuperscript{72,73}. Only few studies have examined HPA axis activity in GAD, reporting increased\textsuperscript{74} or normal cortisol levels\textsuperscript{75-77} and more non-suppression\textsuperscript{71} or normal sup-
pression after dexamethasone. Regarding social phobia, studies showed no difference in basal cortisol levels. Most studies were not able to directly compare different types of disorders or study the effect of comorbid depression. We will contribute to the question which of these anxiety disorders is most strongly associated with HPA axis activity and whether comorbid depression further plays a role.

Previous studies were mostly of small sample sizes, thereby limited in the adequate adjustment for possible confounders and examination of the role of disease characteristics in depressive and anxiety disorders. Also, there is debate about whether observed HPA axis alterations are state or trait dependent, i.e. present only during an episode, or whether they are already present before an episode (vulnerability) or remain present after an episode ('scar'-effect). Furthermore, no previous studies have examined the role of these salivary measures in the course of depressive and anxiety disorders. Finally, the somatic consequences of differences in salivary measures are not fully understood. This thesis aims to contribute to our knowledge of the function of the HPA axis in depressive and anxiety disorders, by addressing certain aspects of this topic which were not covered in previous literature.

THE NETHERLANDS STUDY OF DEPRESSION AND ANXIETY

All the studies in this dissertation were based on data from the Netherlands Study of Depression and Anxiety (NESDA). A full description of NESDA has been provided by Penninx et al. NESDA is a multi-site naturalistic cohort study to: (1) describe the long-term course and consequences of depressive and anxiety disorders, and (2) to integrate biological and psychosocial research paradigms within an epidemiological approach in order to examine (interaction between) predictors of the long-term course and consequences. Respondents were recruited from the general population, general practices and in mental health organizations in order to include persons reflecting various settings and developmental stages of psychopathology. The three main sites were Amsterdam, Leiden and Groningen. During a four-hour baseline assessment, extensive information was gathered about key (mental) health outcomes and demographic, psychosocial, clinical, biological and genetic determinants. The assessment included written questionnaires, interviews, a medical examination, a cognitive computer task, collection of blood and instructions to obtain saliva samples for cortisol measurement at home. Assessed depressive disorders included major depressive disorder and dysthymia. Anxiety disorders that were assessed consisted of GAD, social phobia, panic disorder with and without agoraphobia. In total, 2981 participants aged 18 through 65 years were included. The sample consists of persons with a current or remitted diagnosis of depression and/or anxiety disorder, persons at risk because of a family history or subthreshold depressive or anxiety symptoms, and healthy controls. Its design is an eight-year longitudinal cohort study; detailed assessments will be repeated after one, two,
Introduction

four, six and eight years of follow-up. The findings of NESDA are expected to provide more detailed insight into (predictors of) the long-term course of depressive and anxiety disorders in adults. Besides its scientific relevance, this may contribute to more effective prevention and treatment of depressive and anxiety disorders.

AIMS AND OUTLINE OF THE THESIS

The main objective of this thesis was to examine the role of HPA axis activity in depressive and anxiety disorders. Additional aims were to solve prior inconsistencies through a large sample size, examine other important determinants of salivary cortisol levels, provide more knowledge about the state/trait question, analyze the importance of disease characteristics, examine the role of the HPA axis in the course of depressive and/or anxiety disorders and study the somatic consequences of HPA axis differences. The studies are summarized in Figure 2.

Our first objective was to examine what factors influence cortisol levels in persons without psychopathology to determine which covariates should be taken into account when studying salivary cortisol measures. Chapter 2 describes this study on sociodemographic, health and sampling factors that are considered to affect salivary cortisol. In Chapter 3 we investigate the associations between salivary cortisol indicators and major depressive disorder, taking into account possible confounders and additionally examining the roles of several characteristics of depression. The distinction remitted versus current disorder is made in order to investigate state versus trait effects. Then, in Chapter 4, we examine the relation between salivary cortisol indicators and anxiety disorders, again taking into account remitted versus current disorder, possible confounders and additionally examining the roles of several characteristics of anxiety disorder. Type of disorder, i.e. GAD, social phobia, panic disorder with and without agoraphobia is separately analyzed in relation to salivary cortisol measures, since it is unclear which of the anxiety disorders is most strongly associated with HPA axis activity. In Chapters 5 and 6 we further examine whether cortisol differences are mainly state or trait dependent. In Chapter 5 we compare cortisol levels of persons without psychopathology but with a parent who suffered from depression or anxiety disorder to levels observed in unaffected persons without parental history, in order to examine whether HPA axis differences are present before the onset of depressive and/or anxiety disorders. Furthermore, we relate psychological traits to cortisol awakening levels in persons without psychopathology in Chapter 6. Psychological traits (such as neuroticism, extraversion) that are known to be related with the development of depressive and/or anxiety disorders are examined in relation to salivary cortisol levels without possible (state) effects of psychopathology. Finally, we are interested in the possible psychopathological and somatic consequences of HPA axis dysregulation. Therefore, we conduct a longitudinal study analyzing the associations between salivary
cortisol measures at baseline in persons with a depressive and/or anxiety disorder and the two-year course of these disorders, as Chapter 7 describes. Also, we analyze whether differences in cortisol levels are associated with cardiovascular risk by relating them to (components of the) metabolic syndrome (Chapter 8). In Chapter 9 the results of the studies are summarized and discussed.

Figure 2 Schematic overview of studies in this thesis
References

7. Spijker J, de GR, Bijl RV, Beekman AT, Ormel J, Nolen WA. Duration of major depressive episodes in the general population: results from The Netherlands Mental Health Survey and Incidence Study (NEMESIS). Br J Psychiatry. 2002;181:208-213.