1.1 Introduction

Mental disorders constitute a major burden for society. In 2005, the European Brain Council announced the results of a pan-European project to analyze the prevalence and cost of all mental disorders in Europe (Andlin-Sobocki et al 2005). Across 28 European countries with a total population of 466 million, 127 million people or 27% are affected by at least one brain disease and mental disorders are associated with immense total costs of over 290 billion Euros per year (Wittchen and Jacobi 2005). Mental disorders are complex in their etiology and numerous factors are known to interact in the course of their development. In broad terms it can be stated, that, as proposed by the diathesis-stress model, a genetic vulnerability or predisposition (the diathesis) interacts with the environment and life events (stress) to trigger behaviors or psychological disorders (Zubin and Spring 1977). Indeed, the relationship between mental disorders and stressful life events is well established, both in epidemiological and clinical samples (Paykel 2003), and virtually all psychiatric disorders are closely linked with stress (Young 2004). The question remains, however, why some people who are exposed to an environmental pathogen, e.g. psychological stress, develop mental disorders while others do not. The stress response has evolved as a highly adaptive reaction that ensures survival when an organism is confronted with physiological or psychological challenge. Thus, we are confronted with the dilemma that the same responses, which are adaptive under acute stress conditions, can ultimately promote or sustain disease processes when occurring chronically. Stress physiology has attracted enormous research interest and almost hundred years of investigation have deepened our understanding of the physiological processes, down to the molecular level, elicited under stress. One important stress responsive system is the hypothalamus-pituitary-adrenal (HPA) axis, a hierarchical hormonal system, which mediates the endocrine stress response. This system is under tight self-regulating control through negative feedback mechanisms. The glucocorticoid receptor (GR), scrutinized in this thesis, plays a crucial role in these processes. Interestingly, almost all mental disorders have been shown to be associated with alterations in the HPA axis (McEwen 1998) and these dysregulations are associated with, if not caused by, altered GR functioning. The exact mechanisms how a failure to cope with stress can result in molecular changes and consequently precipitate a disease state are just beginning to be understood. The vulnerable phenotype model proposes that responses to stressors depend on genetic predisposition and are

modulated by the history of the individual, particularly during early life or even prenatally (de Kloet et al 2005).

How can a genetic predisposition be identified? Until the era of molecular genetics, a genetic predisposition was observed when the occurrence of particular disorders tended to run in families. Furthermore, twin studies allowed estimating the heritability, i.e. the part of variance of a trait explained by genetic factors, of personality traits and disease. Thus, the general influence of genetic factors could be estimated, however, these approaches do not allow the identification of the involved, or predisposing, genetic loci. In 2004, following the publication of a rough draft in 2001 (Lander et al 2001), the effort to sequence the human genome was completed and revolutionized medical genetics (International Human Genome Sequencing Consortium, 2004). Not only did the Human Genome Project determine the exact sequence of the human genome and identify the approximately 25,000 genes, more importantly, it provided information about the differences in the genetic makeup of individuals. The human genome has about 10 million polymorphisms, defined as genetic variants in which the minor forms have a prevalence of at least 1% in the population (Goldstein and Cavalleri 2005). The most common type of variants in our genome are single nucleotide polymorphisms (SNPs), the exchange of one base pair through another. Small changes to the genome such as SNPs can exert considerable effects on cellular and tissue level, which can ultimately affect the entire physiology of an organism. SNPs do not invariably *cause* but *predispose* us to common disease, in combination with other genetic variants and the environment we are exposed to. Thus, the main use of a human SNP map will be in dissecting the contributions of individual genes to diseases that have a complex, multigene basis (Chakravarti 2001). Genomic variations are thought to underlie differences in our susceptibility to, or protection from all kinds of disease. In the realm of neuroscience, this knowledge „...promises to provide unprecedented opportunities to explore the genetic basis of individual differences in complex behaviors and vulnerability to neuropsychiatric illness“ (Hariri and Weinberger 2003).

In order to identify individuals at risk for the development of psychiatric disease following stressful events, the underlying genetic architecture of stress-responsive system has to be thoroughly characterized. Experimental work presented in this thesis is intended to contribute to a further understanding of the influence of genetic factors on the functioning of the HPA axis. The aim is to describe the relative contributions of genetic variation of the GR, a key-regulator of this stress-responsive

system, on the regulation of the HPA axis under various stimulation procedures. The characterization of HPA axis response phenotypes in individuals carrying different GR genotypes can be a first step in the identification of individuals who are vulnerable to or protected against the development of stress-related disorders.

1.2 Outline

A consistent feature of HPA axis activity is considerable individual variation in response dispositions (Mason 1968). A number of factors accounting for the observed variability have been identified, including, among others, sex (Kudielka and Kirschbaum 2005), chronic stress (Schulz et al 1998) exposition to early trauma (Heim et al 1998) or maternal prenatal stress (Wadhwa 2005). The question to what extend HPA regulation is influenced by genetic factors has not been studied extensively. Although substantial heritability measures for HPA axis responses have been documented in twin studies (Federenko et al 2004; Wüst et al 2005), the contribution of variation in single genes implicated in HPA axis regulation has not been thoroughly investigated. Genetic variations of the GR are likely to constitute a factor in the observed variability of HPA responses. A large number of polymorphisms of the glucocorticoid receptor gene have been identified, however, the number of variants relevant for the explanation of variance in the general population is likely to be small. Four SNPs of the GR have been studied more or less extensively and associations with measures of body composition, metabolic parameters and indices of GC sensitivity could be revealed (see Chapter 2). Given this evidence, functional relevance of these variants for GC sensitivity seems obvious. Thus, the aim of this thesis is to investigate the influence of all common GR gene polymorphisms with known functionality or previously reported associations and sufficient prevalence in the population (ER22/23EK, N363S, *Bcl*I, 9beta) on HPA axis activity following a psychosocial stressor, sensitivity to exogenous glucocorticoid administration and on working memory performance.

The general introduction in the present **Chapter 1** is intended to explain the rationale of the research strategy underlying the line of work presented in this thesis. **Chapter 2** presents the theoretical background on the topic and is intended to briefly define the term stress and highlight the brain processes involved in the regulation of our organism's stress-sensitive systems. One focus will be on the role of corticosteroid receptors in stress physiology and it will be addressed, how the same

responses that allow adaptation to a stressor can eventually promote disease processes. Emphasize will be put on the role of altered GR signaling in these processes and the molecular mechanism of GR functioning and GC signaling will be described in detail. In **Chapter 3**, HPA axis responses following a psychosocial stress protocol in the different GR genotype groups are presented. **Chapter 4** investigates GR genotype groups with regard to differences in glucocorticoid sensitivity in different tissues, i.e. peripheral leukocytes, subdermal blood vessel and pituitary. In **Chapter 5**, the impact of GR gene polymorphisms on working memory performance under cortisol and placebo administration is scrutinized. **Chapter 6** is intended to highlight availability of glucocorticoids as another key element affecting GC signaling. For this purpose, the effect of corticosteroid binding globulin (CBG), a key regulator of glucocorticoid availability, on HPA axis responses to pharmacological and psychological stimulation is presented. **Chapter 7** provides a general discussion of the findings followed by an outlook in **Chapter 8** where future research directions are delineated.

Chapters 3-6 are written so that they can be read separately, making a certain amount of redundancy unavoidable. These chapters represent manuscript drafts that will be submitted for publication to different journals. Experimental work presented was conducted in collaboration with Prof. Dr. Hellhammer, Dr. Stefan Wüst and Sonja Entringer from the University of Trier and with Elisabeth van Rossum and Jan Willem Koper from Erasmus Medical Center, Rotterdam. Since not all subjects were subjected to every experiment conducted, the number of investigated subjects shows slight variation in the different chapters.

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CHAPTER 2

Theoretical Background

2.1 Stress and the Brain: From Adaptation to Disease

2.1.1 Defining Stress

The term stress was originally taken from the dynamics of physics to describe „the mutual actions which take place across any section of a body to which a system of forces is applied“ (see Levine 2003). Walter Cannon, who first used the stress term in a biological context, defined stress in terms of the stimulus required to elicit adrenomedullary responses (Cannon 1914; Cannon 1915; Cannon 1932). The other pioneer in stress research, Hans Selye, who was also responsible for popularizing the concept in the biomedical community (Sapolsky 1994), defined stress in terms of responses of the endocrine, autonomic and immune system (Selye 1936; Selye 1956). Since then, numerous attempts to define stress have been undertaken, each emphasizing different components. Levine and Ursin (1991) pointed out that the stress concept is a composite and multidimensional concept with interacting subclasses. The three main subclasses can be identified as the stress stimulus (the input), the processing system and the stress response (the output). The stress system affects many physiological processes and “may function as a common alarm and drive system, whenever there is a real or apparent challenge to the self-regulating systems of the organism”.

Whereas Lazarus & Folkman (1984) put emphasize on the transactional element of stress, defining psychological stress as “a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding his or her resources and endangering his or her well-being“, the definition of Chrousos and Gold (1992) has the concept of homeostasis at the core. Homeostasis is defined as a dynamic and harmonious equilibrium that is constantly challenged by intrinsic or extrinsic disturbing forces. Living organisms survive by maintaining this state and this is achieved by adaptational responses consisting of physical and mental reactions that are activated to counteract the effects of stressors in order to reestablish homeostasis. In biomedical research, the concept of stress as a state of threatened homeostasis is the most prominent one and stress “often refers to situations in which adrenal glucocorticoids (GCs) and catecholamines are elevated because of an experience” (McEwen 2000). Taken together, stress can be viewed as an adaptive response of an organism in response to threats of physiological or psychological well-being.

2.1.2 Acute Stress

The effects of stress become manifest in behavior, subjective experience, cognitive function and physiology (Steptoe 2000). There is a surge in arousal, focused attention, vigilance, alertness and cognitive processing. Peripherally, physiological and behavioral responses are triggered aimed at reinstating homeostasis, reflected in activation of the sympathetic nervous system and a rise in GC concentration through activation of the hypothalamus-pituitary-adrenal (HPA) axis. Activation of the HPA axis plays a crucial role in adaptation to homeostatic challenge and GCs are presumed to restore homeostasis following disruption. GCs act at virtually all levels of the body through binding to the glucocorticoid receptor (GR; see below). The end effects of GCs include, among others, energy mobilization, suppression of several immune functions, potentiation of sympathetic nervous system-mediated vasoconstriction and suppression of reproductive function (Sapolsky 2000). Another important function of GCs is the exertion of negative feedback at multiple brain sites to restrain the stress response and adequately control GC secretion (Chrousos and Gold 1992; Jacobson and Sapolsky 1991). These processes are coordinated by distinct stress-responsive systems in the brain and will be described below.

2.1.3 Stress Neurocircuitry

The fact that the triggered responses are both essential for survival and are remarkably consistent in their presentation has led to the suggestion that a discrete neuronal system has evolved for the coordination of the adaptive responses observed under stress (Chrousos and Gold 1992). The two principal components governing the stress response are the corticotropin releasing hormone (CRH) and locus coeruleus-norepinephrine system. In this context, the central mechanisms controlling the CRH system and thereby hypothalamo-pituitary-adrenocortical responsiveness will be highlighted.

The CRH neurons of the hypothalamic paraventricular nucleus (PVN) integrate excitatory and inhibitory inputs into a net secretory signal at the pituitary gland. Release of CRH and the co-expressed neuropeptide vasopressin (AVP) are essential for coordinating the stress response and for governing HPA axis activity. They trigger the release of ACTH from the pituitary, which results in secretion of GCs from the adrenals. The HPA axis has two modes of operation. One is the regulation of the diurnal rhythm of GC secretion and the other is the control of GC secretion following stress. Herman et al. (2003) hypothesize two distinct realms of stress activation.

Stimuli triggering ´reactive´ responses represent genuine homeostatic challenges recognized by somatic or visceral sensory pathways. These stressors would include pain, humoral homeostatic signals (e.g. changes in glucose or insulin levels) or humoral inflammatory signals. These inputs are mediated via direct innervations to the PVN from regions known to receive first- or second-order inputs from somatic nociceptors, visceral afferents or humoral sensory pathways and can therefore elicit rapid and reflexive activation of the HPA axis (see Herman et al 2003). Important for understanding physiological reactions to psychological or psychosocial stress is the fact that activation of the HPA axis can also occur in the absence of physiological challenge. These reactions are termed ´anticipatory´ responses and are centrally generated to mount a GC response in anticipation, rather than in reaction to, homeostatic disruption. Anticipatory responses can be elicited either by classically or contextually conditioned stimuli, i.e. memory programs, or innate species-specific predispositions. These innate programs include the recognition of predators or illuminated spaces for rodents, and also in humans, social challenges and unfamiliar environments or situations. In 1968 John Mason noted: „Psychological influences are among the most potent natural stimuli known to affect pituitary-adrenal cortical activity“ (Mason 1968). Situations characterized by novelty, uncontrollability and unpredictability, perception of threat and ego-involvement are known to reliably elicit HPA axis responses. Anticipatory responses are under control of limbic brain regions, which serve as the interface between the incoming sensory information and the appraisal process. Limbic regions known to influence the stress response include the hippocampus, nuclei of the amygdala, the lateral septum and the medial prefrontal cortex. However, none of these regions send direct projections to the PVN. Modulation of PVN activity is achieved through interactions with ´reactive´ stress circuits in brainstem, hypothalamic regions and regions of the bed nucleus of the stria terminalis (BNST) that directly innervate the PVN. Thus, limbic input is superimposed onto brainstem and hypothalamic stress effectors and a hierarchical system is formed capable of mediating both reactive and anticipatory stress responses.

2.1.4 Dynamics of the Stress Response: Role of Corticosteroid Receptors

Two modes of operation of the stress system have been suggested (reviewed by De Kloet et al. 2005). The system responsible for the initiation of the stress response, the fast mode, involves the above described CRH system, which drives the sympathetic and behavioral ´fight or flight´ response and activates the HPA axis. The

other slower mode terminates the stress response and thus promotes adaptation and recovery. Glucocorticoids operate in both modes through a dual receptor system, which consists of the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Both receptors bind cortisol in humans, albeit with considerable differences in affinity (De Kloet et al 1998; Reul and De Kloet 1985). As the affinity of the MR for cortisol is about tenfold higher than that of the GR, MR activation is maintained even under basal condition whereas the GR becomes activated during stress- and circadian-induced increases in GC concentration (Reul et al 2000). Based on these findings, different roles in HPA axis regulation were suggested for the two corticosteroid receptors: the MR, being occupied to about 80% under basal conditions, was thought to mediate the tonic inhibitory control on HPA axis activity, whereas GR mediate the negative feedback of elevated GC levels (De Kloet and Reul 1987). However, more recent studies indicate that the MR system is not a static system merely playing a cofactor role but rather represents a dynamic system responding to changing requirements, which participates in adaptive mechanisms in the brain evoked by stress. Findings supporting this view were presented, for instance, by Gesing et al. (2001) who report a transient increase in MR density following psychological stress. Furthermore, Cole et al. (2000) reported that administration of MR antagonist, but not GR antagonist, completely blocked habituation of adrenocortical reaction to repeated immobilization stress, supporting the view of a more dynamic role of the MR in the stress response. The notion that the MR is implicated in the appraisal process and the onset of the stress response (de Kloet et al 2005) is supported by findings demonstrating that corticosterone in the rat rapidly and reversibly changes hippocampal signaling through membrane-located MR (Karst et al 2005).

The GR, which becomes activated only by large amounts of GCs, terminates the stress response via the exertion of negative feedback at level of the pituitary, the PVN and at hippocampal sites (Herman et al 2003; Jacobson and Sapolsky 1991; Sapolsky et al 2000). Feedback mechanisms involve genomic DNA binding-dependent and -independent actions as well as rapid nongenomic actions (see section 2.2.1 for details). In summary, GCs in the brain act through two types of corticosteroid receptors allowing differential actions over the time course of the stress response. The MR is mostly responsible for the maintenance of the stress-related neural circuits, whereas the GR is important for the normalization of homeostasis.