1 INTRODUCTION

Different lines of research in animals and humans indicate that adverse prenatal conditions such as malnutrition, stress or anxiety experienced by pregnant mothers can have long-lasting effects on postnatal biological and psychological processes in the offspring.

Numerous studies have provided evidence that maternal stress acts on the fetal HPA axis and may provoke dysregulations of postnatal HPA axis functioning in the child, which, in turn, may have a negative impact on postnatal psychological adaptation. These effects of prenatal stress may result from adverse influences on the fetal physiological system. It is well established that exposure to prenatal stress has programming effects on the brain and the hypothalamus-pituitary-adrenal (HPA) axis of the fetus. The placental enzyme, 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) acts as a protective barrier to maternal glucocorticoids during fetal development. Excess glucocorticoids in early life can permanently alter tissue glucocorticoid signaling. These effects may have short-term adaptive benefits but otherwise increase the risk of later disease.

A variety of naturally occurring stressors provoke maternal hormonal HPA axis activation. However, intra- and inter-individual psychological and physiological reactions are only comparable to a limited extent. Pregnant women who have an imminent risk of a preterm delivery are administered synthetic glucocorticoids for medical reasons. In such cases, prenatally administered synthetic glucocorticoids accelerate fetal lung maturation, which is required for extra-uterine survival. Prenatal glucocorticoid therapy is associated with a decrease in the incidence of respiratory distress syndrome (RDS), and therefore with better newborn survival. Betamethasone belongs to the group of synthetic glucocorticoids that is able to cross the placental barrier and stimulate the fetal HPA axis. Therefore, the application of synthetic glucocorticoids (e.g., betamethasone) acts as a pharmacological stressor. This pharmacological stimulation mimics the effects of a naturally occurring stressor. In addition, it is possible to counteract the problems that arise when comparing the various prenatal stressors.

Despite the growing body of studies in recent years focusing on the effects of prenatal stress in animals and humans, many issues still have to be addressed. In particular, there are gaps in the current knowledge about the association between prenatal glucocorticoid exposure and psychological and biological stress reactivity in humans.

Moreover, so far, few human studies have examined long-term effects on anxiety as a consequence of prenatal glucocorticoid exposure. In particular, knowledge is lacking about children born after the 34th week of gestation without complications (e.g. little administration due to respiratory distress syndrome) as well as with no physical diseases or disabilities after birth. Moreover, to date, the long-

term effects of prenatal glucocorticoid exposure in children who are healthy and show no disparities in their level of development are poorly understood.

Adaptative biological, psychological and social development of children is a crucial condition for adaptation later in life as well as mental and physical health. Consequences of prenatal stress exposure might interfere with these development issues, and therefore have a detrimental impact on children's health.

The present thesis about long-term effects of prenatal stress on psychobiological reactivity to acute psychosocial stress and on anxiety in 10-year-old children includes the following three main parts: a theoretical background, the empirical study, and a general discussion. The theoretical background consists of operationalizations, concepts and empirical studies about prenatal stress (chapter 2.1), psychobiological stress reactivity (chapter 2.2) and anxiety (chapter 2.3). Then, study design, questions and hypotheses will be briefly discussed (chapter 3). Following this, the study examining long-term effects of prenatal betamethasone exposure on biopsychosocial development of children will be presented (chapter 4). The first topic focuses on long-term consequences of prenatal betamethasone exposure on psychological, endocrine, and autonomic responses to a standardized psychosocial stress test (chapter 4.1). The second topic aimed to determine the long-term consequences of prenatal betamethasone exposure on anxiety as assessed by self-report and parental report (chapter 4.2). Finally, a general discussion (chapter 5) will summarize the results of the empirical study (chapter 5.1) and embed the findings into the theoretical background (chapter 5.2), limitations of the study will be discussed (chapter 5.3), and directions for future research will be presented (chapter 5.4). References will subsequently be listed (chapter 6).

2 PART I THEORETICAL BACKGROUND

Prenatal stress has gained growing interest in research in the last years. In the following, an overview of prenatal stress is presented (chapter 2.1), with a particular focus on prenatal glucocorticocid administration. The reported studies yield convincingly evidence that prenatal stress has multifaceted effects on development and behavior later in life. This thesis is focusing of the impact of prenatal glucocorticoid administration on psychobiological stress reactivity (chapter 2.2) and anxiety (chapter 2.3). The presented theories and studies are taken together in the conclusion at the end of the theoretical background (chapter 2.4).

2.1 PRENATAL STRESS

Before going into detail about prenatal stress (chapter 2.1.2), which is the main interest in this thesis, the concept of stress will be discussed first (chapter 2.1.1).

2.1.1 Stress

The definition of stress and theories of stress (chapter 2.1.1.1), biological components of stress – HPA and SNS (chapter 2.1.1.2), as well as the psychological components of stress (chapter 2.1.1.3) and furthermore, first wave and second wave (chapter 2.1.1.4) were discussed.

2.1.1.1 DEFINITION OF STRESS AND THEORIES OF STRESS

Stress is ubiquitous in everyday life. Definitions of the term stress are widespread. According to Selye (1981) stress refers to the consequence of the failure of an human or animal organism to respond appropriately to emotional or physical threats, whether actual or imagined. McEwen (1998b, 2007) defines stress as a threat to the organisms homeostatis. Furthermore, stress can as well be considered as positive, called eustress, if it enhances function (Selye, 1993). Contrary, distress refers to persistent stress that is not resolved through coping or adaptation.

In the following some of the most important concepts like the pioneering work of Cannon are presented. He introduced the term homeostasis that means the sum of regulation mechanism that maintains physiological equilibrium (Cannon, 1932). Threats from outside release a cascade of unspecific, physiological activities that prepare the organism to fight or flight. He observed that on a confrontation with physical or physiological strains the sympathetic nervous system is arousing (Cannon, 1935). According to Cannon, stress is an emergency reaction that in case of any disturbances of the homeostasis, tries to reestablish the equilibrium (Rieger, 2005).

Later, Selye defined the reaction of the organism when physiological homeostatis is treated and described the following three steps (Selye, 1981, 1993). First, an alarm reaction is induced which goes together with an activation of the HPA axis and the SAM system. Second, an resistance phase follows which is characterized by the organism regaining its inner balance (homeostatis). Third, threat is enduring and the regulating mechanisms will exhaust. This phase in which a gradual decrease of resistance to stress occurring is called exhaustion phase. The succession of these three steps Selye referred as general adaption syndrome (GAS). He defined GAS as an unspecific reaction of the organism (Selye, 1981). Mason could show that the physiological response to stress is not uni-dimensional. In animal studies he observed different endocrine reactions under different situational conditions (e.g. fasting, heat, cold). He introduced the significance of psychological stressor in stress research, especially emotional and appraisal components in the stress concept. Only when the physical stressor has a psychological aversive character on a emotional level, it exerts an impact on hormonal level (Mason, 1986).

Important enhancements of the stress concept were made by Lazarus. He postulated a transactional stress model in which the psychological components are central (Lazarus, 1993, 1999) (chapter 2.1.1.3).

McEwen (1998b) presented the concepts of allostasis and allostatic load. Allostasis describes the effort of the body to maintain internal stability. Therefore, adaptation to acute stress is essential for the internal regulation (allostasis) and not generally harmful. A cascade which involves the activation of hormonal stress systems such as the HPA axis is triggered by the process of achieving internal stability. These systems promote organism's adaptation during adaptive short term activation and recovery is provided after cessation of the stressor (McEwen, 1998a, 1998b). The concept of allostatic load refers to the maladaptive consequences of sustained activation of primary regulatory systems over the time. It describes the long term burden resulting from repeated strain. Allostatic load contributes to organ damage and disease (McEwen, 1998a, 1998b). Moreover, McEwen's concept provides a potential explanatory approach for a range of pathological mental and physical conditions (De Kloet, Joëls & Hoslboer, 2005).

2.1.1.2 BIOLOGICAL COMPONENTS OF STRESS – HPA AND SNS

When the brain detects a threat, a coordinated physiological response involving neuroendocrine, autonomic, metabolic as well as immune system components are activated (Lupien, McEwen, Gunnar & Heim, 2009). Key systems in the stress response are the hypothalamus-pituitary-adrenal (HPA) axis and the autonomic nervous system, in particular the sympathico adrenal medular system (SAM) (the sympathetic response of the of the adrenal medulla oblongata and sympathetic nerves) (McEwen, 2000).

Hypothalamus-pituitary-adrenal (HPA) axis. Following a stressful event subsequent cascade occurs. Neurons in the medial parvocellular region of the paraventricular nucleus of the hypothalamus release corticotropinreleasing hormone (CRH) and arginine vasopressin (AVP). This triggers the subsequent secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland, leading to the production of glucocorticoids (cortisol) by the adrenal cortex (Lupien et al., 2009). The responsiveness of the HPA axis to stress is in part determined by the ability of glucocorticoids to regulate ACTH and CRH release by binding to two corticosteroid receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Following activation of the system and once the perceived stressor has subsided, feedback loops are triggered at various levels of the system: from the adrenal gland to the hypothalamus and other brain regions such as the hippocampus and the frontal cortex (Heim & Meinlschmidt, 2003). Consequently, the HPA axis is shut down and returns to a set homeostatic point.

The sympathetic nervous system (SNS). The autonomic nervous system modulates the activity of the body's organs, regulating cardiovascular, respiratory, gastrointestinal, electrodermal, endocrine as well as exocrine functions (Pinel & Pauli, 2007). It is divided into two subsystems: sympathetic nervous system and the parasympathetic nervous system (PNS). Fibers from the SNS innervate tissues in many organ system. Thereby, providing at least some regulatory function mediating the neuronal and hormonal stress response known as the *fight-or-flight response or sympatho-adrenal response* of the organism (Heim & Meinlschmidt, 2003). The preganglionic sympathetic fibers that end in the adrenal medulla secrete acetylcholine. Subsequent, adrenaline (epinephrine) and to a lesser extent noradrenaline (norepinephrine) are secreted. This response acts primarily on the cardiovascular system and is mediated directly via impulses transmitted through the sympathetic nervous system and indirectly via catecholamines secreted from the adrenal medulla (Pinel & Pauli, 2007).

In research, salivary cortisol is routinely used as a biomarker of psychological stress (Hellhammer, Wust & Kudielka, 2009). Most studies consider salivary cortisol levels a reliable measure of hypothalamus—pituitary—adrenal (HPA) axis adaptation to stress. The HPA axis maintains the organism's capacity to respond to acute and prolonged stressors and is a focus of research on the sequelae of stress (Gunnar, Talge & Herrera, 2009).

Cortisol. Cortisol is a glucocorticoid hormone that is synthesized in the adrenal zona fasciculata (Heim & Meinlschmidt, 2003). The small lipophilic molecule is based on cholesterine, enzymatically restructured in several steps and delivered into blood stream (Kolb & Whishaw, 2001). It is able to diffuse in all body fluids: urine, liquor, sperm, sweat as well as saliva. For cortisol, two kind of receptors have been identified: the glucocorticid receptor (GR) and the mineralcorticoid receptor (MR) (De Kloet, 2003). Cortisol demonstrates a strong diurnal and circadian variance due to time-dependent activity patterns of the HPA axis (Heim & Meinlschmidt, 2003).

Salivary alpha-amylase (sAA). Salivary alpha-amylase release is known to be elicited by activation of the autonomic nervous system (ANS) which controls the salivary glands (Nater & Rohleder, 2009). It is one of the most important enzymes in saliva. Alpha-amylase hydrolyzes starch to glucose and maltose, thereby initiating the digestion of starch in the oral cavity. Furthermore, sAA has a bacterial interactive function (Scannapieco, Torres & Levine, 1993). Salivary alpha-amylase has been proposed as a sensitive biomarker for stress-related changes in the body that reflect the activity of the interplay between SNS and PNS (Nater & Rohleder, 2009). A growing body of research is accumulating to support the validity and reliability of this parameter (Chatterton, Vogelsong, Lu, Ellman & Hudgens, 1996; Nater et al., 2005, 2006).

Heart rate. The heart is innervated by the autonomic nervous system (Pinel & Pauli, 2007). The cumulating influences of the sympathic and the parasympathic branches are reflected in the heart rate (Bauer, Quas & Boyce, 2002). The adrenergic influence of the sympathicus increases heart rate, whereas the cholinergic parasympathetic influence of the vagus nerve reduces it (Schandry, 2006). During rest, heart is primarily under vagal influences. This vagal tonus is attenuated with increasing strain / stress by the dorsal motor vagus nucleus of medulla oblongata (elongated spinal cord) (Vossel, 1998). Heart rate (HR) means the number of heart beats per time unit and is typically expressed as beats per minute (BPM). If the heart rate is too fast it's called tachycardia. Whereas, bradycardia means a too slow heart rate (Schandry, 1998).

2.1.1.3 PSYCHOLOGICAL COMPONENTS OF STRESS

As mentioned above, Lazarus postulated a transactional stress model in which the psychological components are central (Lazarus, 1993). In this model, stress is understood as an imbalance occurring between prevailing demands and individual resources to cope (Lazarus, 1986; Lazarus & Folkman, 1984). According to Lazarus (1986) "Psychological stress is 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" (S.19).

Following this model, the cognitive process consists of a primary and a secondary appraisal (Lazarus & Folkman, 1984). A person perceives a potential stressor and interprets a situation as: irrelevant, positive or harm, loss, threat or a challenge (Folkman, 1997).This process is called primary appraisal. During the secondary appraisal, alternative solutions as well as perceived coping abilities and consequences of a potential action were considered.

In addition, the concept of allostasis and allostatic load also accounts for psychological factors (beside the effect of stress on physiological processes), it's proposing that cognitive processes moderate the stress response and explain the existence of interindividual differences (McEwen, 2007; 2008).

2.1.1.4 FIRST WAVE AND SECOND WAVE

The stress response activates two pathways (Kolb & Whishaw, 2001) a slow-acting and a fast-acting pathway (Sapolsky, 2000; Sapolsky, Romero & Munck, 2000). In the following both biochemical sequences are summarized in brief (Becker et al., 2000).

Slow-acting pathway. In the brain, the hypothalamus releases corticotrophin-releasing hormone (CRH) through veins into the anterior pituitary. This gland releases adrenocorticotropin-releasing hormone (ACTH) into the bloodstream. ACTH acts in this way on the adrenal gland. The adrenal cortex releases cortisol into the circulatory system.

Fast-acting pathway. The brain signals the spinal cord thereby activating the sympathetic system of the spinal cord that stimulates the adrenal gland. From the adrenal medulla is adrenaline released into the circulatory system.

Both, adrenaline and cortisol activates endocrine glands, body cells as well as the brain and thereby reducing and controlling the stressor (Kolb & Whishaw, 2001). The adrenalin pathway is activated fast (sec) and prepares the body for a sudden burst of activity. Whereas, the cortisol pathway is activated slow (min to hours) thereby preparing the body for longer-lasting adaptations (e.g. restoration of cells after energy expenditure).