1. Introduction

The assumption that maternal stressful experiences during pregnancy may have long lasting effects on the developing foetus is ancient (Schlotz & Phillips, 2009). Stress research has taken up this assumption in the middle of the last century and begun to investigate it more rigorously over the past 20 years. In the meantime, animal studies have long proven that maternal stress can adversely affect the offspring's development, enhancing the likelihood of poor pregnancy outcome (Brunton & Russell, 2011; Weinstock, 2008). Prenatal stress in animals has been associated with a wide range of problems for the offspring such as low birth weight, a deregulated hyperactive hypothalamic-pituitary-adrenal (HPA) axis, adult hypertension, impaired cognitive and memory functioning as well as increased anxiety and depressive-like behaviour (Brunton & Russell, 2011; Seckl, 2004; Weinstock, 2005, 2008). Similar results are beginning to emerge from human studies (Glover, O'Connor, & O'Donnell, 2010). Such consequences of prenatal stress have also been termed *fetal programming effects* (Barker, 1998; Drake, Tang, & Nyirenda, 2007). The concept of *fetal programming* states that there are critical periods during intrauterine development in which external stimuli or negative events may have a lifelong lasting impact on the foetus. However, the path by which maternal stress may reach the unborn still remains puzzling, since there are no neuronal connections between mother and foetus. How then can something that is not even material but rather psychological affect the unborn child? Different hypotheses exist and are subject of intense investigation. Nevertheless, before clear statements can be made a better understanding of the pregnant woman's stress experience, the manner in which she reacts psychologically as well as physiologically to stress is necessary.

In this thesis the focus is laid on human pregnancy and therefore most reported findings are based on human studies unless otherwise indicated. The theoretical background (Part I) of the empirical studies (Part II) is presented in Chapter 1 in which a general overview on the psychobiology of stress, the definition of stress and its related concepts and the psychobiological stress response in the non-pregnant state is described. In this regard, two of the main stress responsive systems, namely the HPA axis and the autonomic nervous system (ANS) are introduced. The changes that these two stress systems undergo during pregnancy are discussed in Chapter 3. Chapter 4 briefly summarizes various stress settings that have been investigated by pregnancy research. Subsequently, findings on how pregnant woman

respond to stress in general are reviewed focusing on whether this differs from the non-pregnant state and whether there are any periods during pregnancy which are especially vulnerable to stress. Finally, Chapter 5 is dedicated to the question of how maternal stress may arrive at and impact the unborn child. In this regard, the activity of a specific placental enzyme is highlighted, which has been proposed to play a vital role in protecting the foetus from excessive maternal stress hormones.

After presenting the empirical study findings in Part II, the results are discussed in more detail in the general discussion in Part III.

PART I THEORETICAL BACKGROUND

2. The Psychobiology of Stress

Stress scientists have become increasingly aware of how psychological factors influence human health and disease. This is also the case during pregnancy except that for the duration of this condition stress not only may affect the mother but also the developing foetus. A growing body of research has found increased risk of pregnancy loss, preterm delivery and low birth weight to be associated with psychological stress (Dunkel Schetter, 2011; Madhappan et al., 2003; Mulder et al., 2002). Therefore, in order to protect the pregnant mother and her foetus from the detrimental effects of stress, it is essential to understand what stress is, which situations induce stress and whether all forms of stress are harmful.

2.1 The Definition of Stress and Its Related Concepts

In 1973, the endocrinologist Hans Selye stated that "*everybody knows what stress is, and nobody knows what it is"* (Selye, 1973, p. 692). Although knowledge has much increased since then through research, this statement is still relevant today. The phenomenon of stress in everyday life is of great interest to scientists from countless scientific fields, yet, consensus on a definition is lacking (Koolhaas et al., 2011). The Oxford Online Dictionary defines stress as *"pressure or tension exerted on a material object"* or *"a state of mental or emotional strain or tension resulting from adverse or demanding circumstances"* (Oxford Online Dictionary, 2011). The term originates from the Latin verb, *stringere,* meaning to draw tight.

Our understanding of stress has evolved greatly over past decades. The notions of *equilibrium* as a state of health, and *imbalance* as the source of disease dates back to the ancient Greeks and Romans (Chrousos & Gold, 1992). In the 19th century this concept was developed further by the research of the French physiologist, Claude Bernard (Cooper, 2008; Goldstein & Kopin, 2007), who discovered, among other things, that the vascular blood flow is regulated by sympathetic nerves. His work led him to the concept of the *milieu intérieur,* meaning that the internal environment of the body made of fluids such as blood and lymph surround the body tissues and organs (Cannon, 1929). Bernard formulated the theory that, in response to disturbances from the external environment and in order to maintain physiological

equilibrium, the *milieu intérieur* is regulated dynamically by the nervous system (Chrousos & Gold, 1992; Cooper, 2008). This equilibrium is not a default condition but rather a result of continuous change and regulation.

Inspired by Bernard, the American physiologist Walter B. Cannon espoused the idea of the milieu intérieur and coined the term *homeostasis*. Cannon (1929) explained that he chose the Greek-derived prefix *homeo* (meaning "like" or "similar") instead of *homo* (meaning "same") because the later would have implied something rigid and fixed. In animal studies, Cannon observed that emotions such as fear, rage and pain lead to rapid activation of the sympathetic nervous system and secretion of epinephrine from the adrenal medulla (Cannon, 1914). According to Cannon, these two important protagonists, the sympathetic nervous system and the adrenal medulla, operate as a unit to restore homeostasis. He referred to this system as the *sympathico-adrenal system* (Cannon, 1929; Goldstein & Kopin, 2007).

Influenced by the British social psychologist, William McDougall, Cannon interpreted his findings as an emergency response of the nervous system induced by these strong emotions in order to supply the organism with enough energy to either *fight or flee* (Cannon, 1914; Cooper, 2008). This response of the sympathetic nervous system was therefore termed the *fight-or-flight* response (Iversen, Iversen, & Saper, 2000). Cannon's co-workers later introduced the concept of *negative feedback*, a term originally derived from engineering that describes an important mechanism to safeguard homeostasis (Cooper, 2008; Rosenblueth, Wiener, & Bigelow, 1943). They defined *negative feedback* as corrective information acquired from the *output* or also response of a living organism (Rosenblueth et al., 1943).

In 1936, the endocrinologist, Hans Selye, used the word *stress* which was derived from physics (Chrousos & Gold, 1992; Goldstein & Kopin, 2007) and defined it as *"a nonspecific response of the body to any demand upon it"* (Selye, 1973, p. 692). A stressor was defined as an agent that causes stress. Selye's concept of a *"nonspecific response"* was based on his observations that a variety of stressors such as cold, heat, burns, infections, fasting, trauma etc. each elicited the same physiological response pattern (Mason, 1971; Selye, 1973, 1976).

This response was termed the *General Adaptation Syndrome (GAS)* which develops in three stages: 1) alarm, 2) resistance, and 3) exhaustion (Selye, 1950). In the alarm stage the biological response is distress. In the resistance stage the body resists the imbalance and strives to adapt and regain a homeostatic equilibrium. With continued exposure to the stressor the organism enters the third stage of exhaustion that increases susceptibility to disease. Whilst Cannon's research put emphasis on the activation of the sympathoadrenal system in response to stress, Selye's work emphasized the activation of the hypothalamic-pituitary-adrenal (HPA) axis. Selye also coined the term *eustress* from the Greek word, *eu,* meaning good (Chrousos & Gold, 1992; Lazarus, 1993). He thereby introduced the notion that stress is not always negative or harmful, but that mild forms can have positive effects such as enhanced emotional and intellectual development. Selye's concept of stress generated much interest but also controversy. Although Selye's assumption of nonspecificity has in the meantime been refuted (Mason, 1971; Pacak et al., 1998), his findings concerning stress and the related adverse effects on physical health have become a widely investigated field of research.

Even though Selye acknowledged that psychological stimuli are capable of eliciting a stress response, his focus remained primarily on the impact of physical stimuli. However, the American psychiatrist and psychendocrinologist, John W. Mason, discovered in animal studies that stressors, such as cold, heat, fasting, etc. that were examined by Selye and postulated to cause a nonspecific physiological response, had powerful psychological components as well (Mason, 1971). When these psychological factors were taken into account and were investigated more closely, it became clear that they were the principle causes for the endocrine response of the HPA axis and not merely the physical factors. In Mason's research fasting caused an increased response of the HPA axis in monkeys – but only when the monkeys were surrounded by other monkeys that received feeding, and not when they were sheltered in small cubicles without social contact and the possibility of making social comparisons. Similarly, only a sudden rapid rise of room temperature elicited an HPA axis response in monkeys, but not a gradual one. Likewise, Cannon and his team had noticed that it was primarily the experience of fear that activated a physiological stress response (Cannon, 1914). These findings suggest that stress mainly results from the way an individual perceives and *appraises* a stressor.

From the 1950s on, the role of *appraisal* in the stress response was investigated extensively by the American psychologist, Richard Lazarus, and his colleagues (Lazarus, 1993). For Lazarus (1993) *appraisal* was a mediating process between the demands of the environment and the beliefs and motivations of an individual. Therefore, individual differences in cognitive and motivational aspects became a primary focus of his investigations. He and his work group (Speisman, Lazarus, Mordkoff, & Davison, 1964) studied the reactions of study participants to a silent movie that showed a brutal coming of age ritual in an indigenous Australian tribe. Depending on the manner in which the narrator introduced the movie to the spectators, they exhibited either a stress response (when the brutality was emphasised) or not (when the film was introduced as an anthropological study and the brutality of the ritual was downplayed).

In 1984, Lazarus and his assistant, Susan Folkman, published a *Transactional Model of Stress* – transactional because the model proposed that stress resulted from a transaction between an individual and his or her environment (Lazarus & Folkman, 1984). According to this model, individuals evaluate the situations they are confronted with according to relevance and stressfulness. There are generally three stages in this process. The first is called *primary appraisal*. A situation is appraised by an individual as stressful and relevant when it is perceived as either harmful (e.g., connected with an irrevocable loss), threatening (e.g., harm is impending or anticipated), or challenging (e.g., an individual feels he or she can manage a difficult situation by activating coping strategies). In a second stage termed *secondary appraisal*, an individual instantly analyses whether coping resources are available. Depending on this analysis, a stress response is initiated or not. In the third stage, an individual *re-appraises* the situation based on the new experiences gained.

A more recent concept of stress termed *allostasis* was introduced by the American neuroscientists, Peter Sterling and Joseph Eyer towards the end of the 20th century (Goldstein & Kopin, 2007; Sterling & Eyer, 1988). The concept was further elaborated by Bruce McEwen and other researchers (Goldstein & McEwen, 2002; McEwen, 1998). Allostasis is defined as "*the ability of the body to achieve stability through change"* (Goldstein & McEwen, 2002; McEwen, 1998; Sterling & Eyer, 1988). The term is put together from the Greek prefix *allo* meaning change and *stasis* meaning stable (Schulkin, 2011). According to McEwen (1998) a stress response is triggered when an individual perceives a stressful

situation that involves the experience of threat and helplessness, for instance during a traumatic event. Stressors may also be related to the home, workplace or neighbourhood. They can be real or imagined (Goldstein & Kopin, 2007; McEwen, 1998). The individual's perception of stress depends on his or her genes, previous experiences and personal development (McEwen, 1998). In stressful situations physiological (e.g. activation of the HPA axis, ANS, etc.) and behavioural responses (e.g. fighting or fleeing, changing dieting habits, etc.) are triggered, leading to allostasis and adaptation.

Chronic activation of the allostatic system, however, can result in allostatic load. McEwen (1998) differentiates between four types of allostatic load: 1) repeated stress "hits", 2) responses without adaptation resulting in prolonged exposure to the stressor, 3) the inability to shut the stress response down and recover, and 4) inadequate response. In contrast to homeostasis where the organism depends on negative feedback to maintain equilibrium, the concept of allostasis proposes that negative feedback alone does not suffice the needs of the organism and is too slow to regulate quick responses within the body. In order to effectively adapt to change, the anticipation of change is necessary (Schulkin, 2011; Sterling, 2011). Allostasis therefore depends on anticipatory and consequently feed-forward regulation.

The concept of stress has come a long way. But interest in the subject from innumerable scientific fields is still rapidly growing and, with it, our understanding of this important aspect of human life will surely grow in fast progress as well. The following chapter is dedicated to the psychobiological response to stress.

2.2 The Psychobiological Stress Response

One of the central structures of the brain involved in processing stress-relevant stimuli is the limbic system, which is composed of various brain structures such as the amygdala, the hippocampus and the prefrontal cortex (Gunnar & Quevedo, 2007; Kaltsas & Chrousos, 2007; Shekhar, Truitt, Rainnie, & Sajdyk, 2005). Further central structures responsible in orchestrating the stress response are the brain stem and the hypothalamus (Ulrich-Lai & Herman, 2009). In the body's periphery, the two major systems regulating the stress response are set into motion: the hypothalamic-pituitary-adrenocortical (HPA) axis and the autonomic nervous system (ANS) together with its two branches, the sympathetic nervous

system (SNS) and parasympathetic nervous system (PSNS). On closer inspection, information signalizing a threat (real or potential) is conveyed to the brain stem and/or limbic structures. Bottom-up signals from the brain stem reach the paraventricular nuclei (PVN; see Figure 1) of the hypothalamus through direct projections (Ulrich-Lai & Herman, 2009). The hypothalamus is located above the brain stem and composed of neuron clusters, such as the PVN (Snowdown & Ziegler, 2007). Distinct neurons within the PVN called parvocellular neurons are to a large extent responsible for coordinating the stress response of the HPA axis and ANS (Gunnar & Quevedo, 2007; Ulrich-Lai & Herman, 2009). The structures of the limbic system are indirectly connected with the PVN via top-down signalling and are not only involved in triggering the stress response but also in modulating its further course (Gunnar & Quevedo, 2007; Shekhar et al., 2005). Therefore, these stress responsive systems do not exert their actions separately from each other but are interconnected, each influencing the activity of the other (De Kloet, Vreugdenhil, Oitzl, & Joels, 1998; Gunnar & Quevedo, 2007; Ulrich-Lai & Herman, 2009).

The stress responses of the HPA axis and the ANS are geared towards mobilization of energy in order to deal efficiently with the threat and restore equilibrium. Increased blood flow and oxygen are redirected to the brain, heart and lungs, blood pressure rises, respiration and heart rate speed up, glucose mobilization from the liver increases while immune-mediated inflammation, reproductive hormones, pain sensitivity, bone and muscle growth are suppressed (Cannon, 1914; Ehlert, 2011; Herman et al., 2003; Kaltsas & Chrousos, 2007). Vigilance, alertness and other cognitive functions are enhanced and feeding and sexual behaviour are interrupted (Chrousos & Gold, 1992; Sapolsky, Romero, & Munck, 2000). The pathway of the HPA axis and the ANS activation will be discussed in detail in the following.

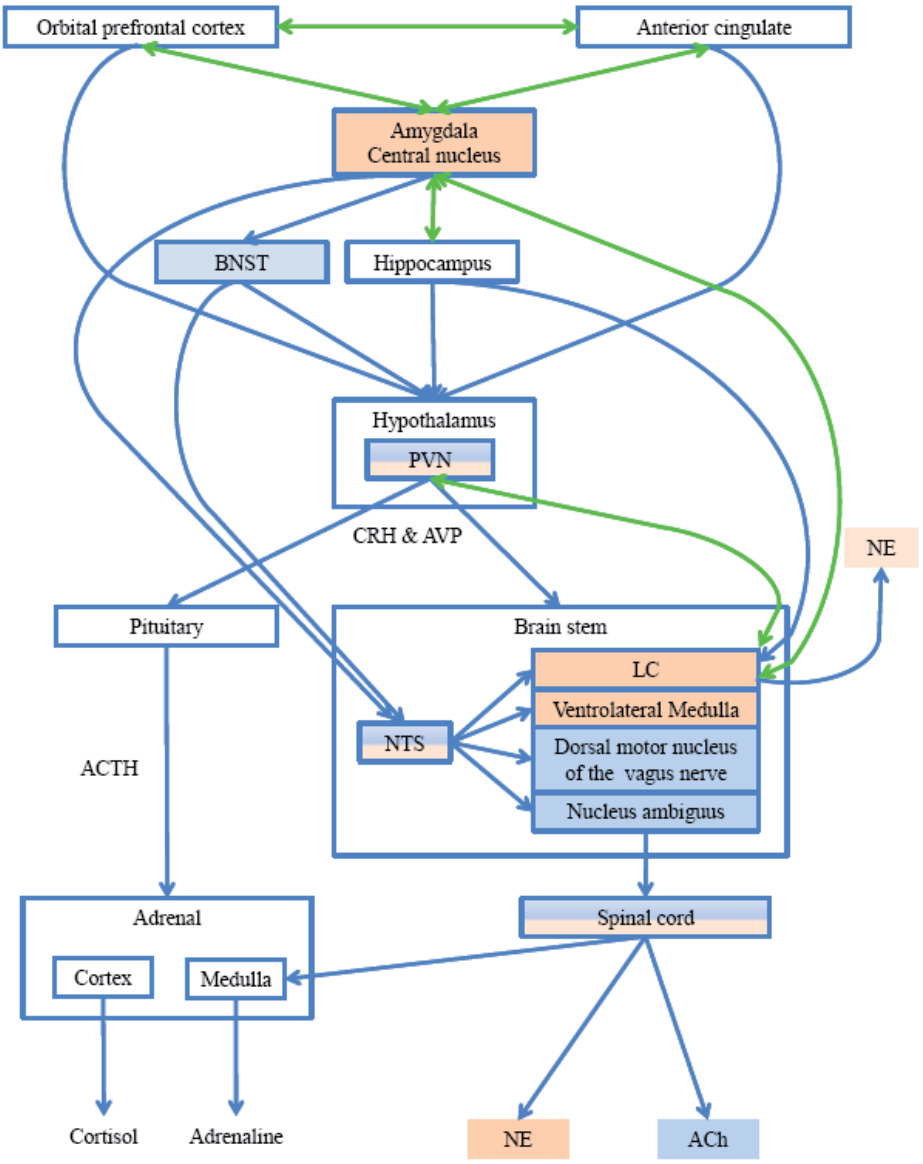

Figure 1. Central structures that regulate the stress response of the HPA Axis and ANS. Brain structures that regulate the sympathetic action are in red and brain structures that regulate the parasympathetic action are in blue. (Adapted from Ulrich-Lai & Herman, 2009 and from Gunnar & Quevedo, 2007).

2.2.1 The Hypothalamus-Pituitary-Adrenal Axis

The activation of the HPA axis (see Figure 1) is triggered by the release of two peptides known as corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP)